The
VETERINARY RECORD
Founded by William Hunting, F.R.C.V.S., in 1888
7, MANSFIELD STREET, PORTLAND PLACE, LONDON, W.1. MUSEUM 6541

No. 42 OCTOBER 16th, 1954 Vol. 66
Registered for Transmission at a Newspaper *Price : 2s. post free*

Contents

General Articles :

A Survey of Canine Distemper (Part II) 623
I. M. Lauder, M.R.C.V.S.,
W. B. Martin, M.R.C.V.S., D.V.S.M.,
Edith B. Gordon, M.R.C.V.S.,
D. D. Lawson, M.R.C.V.S.

Contribution to the Early Embryology of the Sheep: An Attempt to Correlate the Developmental Stage of the Descending Ovum with the Place of Recovery ... 632
R. Hadek, DR. VET. MED. (VIENNA).

Clinical Communication :

Prevention and Treatment of Sepsis in the Bovine Uterus with Bis - parachlorphenyldiguanidohexane 635
J. McWilliam, M.R.C.V.S.

News and Comment 639

Correspondence :

A Legitimate Grievance ... 641
Arthur Welch, M.R.C.V.S.

Gamma Globulin Prophylaxis of Canine Virus Distemper ... 641
J. M. Mackay, M.R.C.V.S.

Cortical Activity in Electrocuted Dogs 642
Phyllis Croft, PH.D., M.R.C.V.S.

Outbreaks of Swine Fever on the Earl of Shrewsbury's Estate ... 642
H. Enoch

A Survey of Canine Distemper

BY
I. M. LAUDER, W. B. MARTIN, EDITH B. GORDON, D. D. LAWSON
(Departments of Veterinary Medicine and Surgery)
and
R. S. F. CAMPBELL and A. M. WATRACH
(Department of Veterinary Pathology)
University of Glasgow Veterinary School

PART II : PATHOLOGY

Morbid Anatomy

MOST of the dogs were examined by autopsy within six hours of death.

The principal gross changes were seen in the thoracic organs. Catarrhal, even purulent, inflammation of the naso-pharynx was fairly common (*vide* Part I : Clinical) and congestion of the submucosal vessels of the pharynx, larynx and trachea often occurred. The bronchi in most cases contained a thin transparent oedematous fluid, almost colourless and often foamy. Less frequently the bronchi contained catarrhal fluid but a frankly purulent type of exudate was present in only a few cases. A state of oedema of the lungs could be recognised macroscopically in nearly all animals and was sometimes so marked that large amounts of serous fluid issued from every part when the tissue was cut and squeezed. The lungs usually showed congestion but haemorrhage was less common. In the early stage of pneumonia the underlying cellular changes combined with the oedema to produce an increased consistency in parts of most of the lobes and the affected areas sometimes had a dull magenta colour which tended to turn reddish-brown on exposure to air. Occasionally both lungs were uniformly firm and swollen so that each of their lobes displayed rounded edges. A feature sometimes seen was consolidated tissue to a depth of several millimetres under the pleura of one or more lobes and varying from small areas of a few millimetres in extent to a diffuse sheet. Six dogs showed distinct broncho-pneumonia or suppurative softening of bacterial origin (four other cases of progressive bacterial infection were detected histologically). The pericardial sac and, to a lesser degree the pleural cavity, contained a slight or moderate excess of serous (rarely sero-haemorrhagic) fluid but fibrin was seldom found. Paleness of the myocardium had often developed and the coronary vessels were at times congested. Subepicardial petechiae were occasionally seen. The mediastinal lymph nodes displayed changes, the severity of which corresponded to the stage of inflammation in the lungs. Acute lymphadenitis was characterised by congestion of the

nodes, swelling and oedema, while in later phases the nodes tended to become firmer and greyish as a result of the regeneration of lymphoid tissue (*vide infra*). Severe oedema and petechiation of the mediastinal connective tissues and thymus were encountered occasionally. Changes similar to those in the thoracic nodes occurred in other lymph glands and in the tonsils though not always to the same degree.

The liver was often slightly paler than normal with a yellowish tinge and especially in the chronic cases showed also centrilobular congestion (nutmeg liver). In some dogs the organ was uniformly congested and darkened. The gastro-intestinal tract was sometimes slightly congested and its contents occasionally blood-tinged. Intestinal nematodes and cestodes, though fairly common, were seldom present in large numbers. Definite pathological enlargement of the spleen was recognised in a few dogs only, when it was associated with roundness of the edges of the organ and a greyish colour resulting from the hyperplastic changes in progress. The appearance of the kidneys varied greatly. While rarely altered appreciably in size they were sometimes rather pale and softened from degenerative change, sometimes dark red from congestion. In other cases no gross abnormalities were detected in them. The wall of the urinary bladder occasionally contained petechial or ecchymotic haemorrhages but such changes, like hyperaemia, could not always be interpreted as either pathological or agonal. The principal gross findings in the central nervous system were hyperaemia of the meningeal and deep vessels, a variable excess of cerebrospinal fluid, sometimes blood tinged, and a gelatinous fluid which often accumulated in the subarachnoid space especially in the region of the chiasma and around the third and fourth ventricles.

Abnormalities of the eyes, nose, skin and pads have been described in Part I.

Histopathology

Technical Methods.—Except for the pads which were fixed in formal-picric acid and treated by the double embedding technique, the tissues were fixed in formal-corrosive sublimate before paraffin embedding. In some cases a portion of cerebellum was treated with formal-ammonium bromide.

All tissues were stained by the haemalum-phloxin-tartrazine method of Lendrum for the demonstration of histological changes in general and cytoplasmic inclusion bodies in particular. Other techniques employed from time to time included Gram-Weigert and Gram-Twort for bacteria, the picro-Mallory method for collagen, the methods of Gordon & Sweets and of Laidlaw for reticulin, Loyez's method for myelin, and phosphotungstic acid-haematoxylin to demonstrate fibrin and neuroglial fibres. In a few cases, axons were impregnated with silver by Bielschowsky's method in frozen sections of formal-fixed tissue. Cajal's gold sublimate was used occasionally to demonstrate astrocytes. The Feulgen technique was employed to identify desoxyribonucleic acid.

The microscopical examination generally included lung, bronchial lymph node, heart, liver, spleen, kidney, urinary bladder, pads (carpal and digital), central cerebral (motor) cortex, cerebellum and pons. In the latter half of the survey the investigation was extended to include tonsil, tongue, duodenum, pancreas and mesenteric lymph node, the optic tract, premotor cortex, basal ganglia, occipital cortex, hippocampus, thalamus, mid-brain and medulla. The spinal cord was studied at six levels, viz., the upper and lower thirds of the cervical, thoracic and lumbar areas in 29 dogs. Other tissues occasionally examined included nasal epidermis, conjunctiva, trachea, oesophagus, stomach, adrenal, thymus, pituitary, and lymph nodes additional to those of the thoracic cavity.

Lung

Hyperaemia and oedema of the alveoli and interstitium were present in almost every case. Mononuclear cell infiltration occurred around the smaller bronchioles (Fig. 8). In many cases alveoli showed a distinct pavement of flattened cells (Fig. 9) which often could be traced directly to the epithelium of the terminal respiratory bronchioles. Such alveolar "epithelialisation" tended to become diffuse subpleurally (Fig. 10) Multinucleated and bizarre forms of cells were sometimes quite common at the height of this process and they were joined by numbers of intra-alveolar macrophages. Smaller numbers of lymphocytes entered the alveoli. In most cases polymorphonuclear leucocytes were scanty, which suggested that bacterial activity was not constant. As the acute visceral phase of distemper subsided, usually after five or six weeks of clinically detectable illness, "epithelialisation" disappeared with desquamation and degeneration of the cells. The intra-alveolar content of cells of all types diminished unless bacterial infection continued. In time few macrophages remained in the air sacs and only small accumulations of lymphocytes, macrophages and plasma cells were found in and around bronchiolar walls or in perivascular sites especially in relation to venules (Fig. 11). The oedematous state of the lungs was still seen in prolonged cases, without appreciable coagulation or fibrin formation.

When secondary bacterial infection did evolve, polymorphonuclear invasion of alveoli, bronchial passages and interstitial tissue was found and in the severest cases softening of the greater part of a lobe resulted.

In all but nine of the 50 cases examined cytoplasmic inclusion bodies (Figs. 12 and 13) were observed in the lung. Eight of the negative cases had clinical illnesses of six weeks or more and although the ninth dog was observed to be ill for only 14 days, the general microscopical picture suggested that infection may have been as prolonged as in the others. Inclusions occurred principally in the bronchial epithelium and alveolar lining cells and in the fixed or free macrophages. Less often they were found in endothelium, polymorphonuclear leucocytes and lymphocytes.

Myocardium

The musculature of the heart usually showed cytoplasmic granularity and lack of definition of the striations. Interstitial oedema and petechiation were occasionally detected, but only in a few animals were cellular inflammatory changes found, in the form of small groups of mononuclears and polymorphonuclears. Focal necrotic lesions containing toxoplasms were present in the heart of one animal. On rare occasions cytoplasmic inclusions were seen in histiocytes.

Lymphoid Tissues

(Nodes, spleen and tonsils). These tissues are dealt with together since identical changes occurred throughout the lymphoid areas. During the acute phase of the infection lymphocytes were considerably depleted and among the remaining cells degenerative changes such as pyknosis and karyorrhexis were commonly seen. As the lymphocytes disappeared the follicular pattern of the lymphoid areas was lost, in the nodes especially, leaving a looser pulp which was often bathed in the excess of lymph and associated with hyperaemia or even haemorrhage. The reticulum cells hypertrophied and this change, together with the lymphoid depletion, rendered them very prominent. It is still not clear whether an increase of reticulum cells takes place; mitotic figures were not observed in them during this phase. In all cases studied in the acute stage a proportion of reticulum cells showed margination of nuclear substance and the formation of a distinct intranuclear mass which stained dull purplish in haemalum-phloxin-tartrazine preparations. These were Feulgen-negative unlike the Feulgen-positive intra-nuclear inclusions of contagious hepatitis to which they otherwise bear some resemblance. Phloxinophilic cytoplasmic inclusions were also found in the reticulum cells, macrophages and less frequently in other cells. The macrophages showed proliferative and degenerative changes and were found in increased numbers in the sinuses. When haemorrhage had occurred, erythrophagocytosis and haemosiderosis developed, the latter being outstanding in the spleen in the late stage. Multinucleated cells with closely packed nuclei were seen in many of the lymph nodes examined in the earlier phase of infection.

It appeared that in the later stage of the infection, probably about five weeks from the onset, signs of regeneration of lymphoid tissue occurred in the form of new follicles in which lymphoblastic as well as lymphocytic cells were prominent. The number of mitotic cells increased. The medullary cords in the nodes were also hyperplastic and sometimes contained very numerous plasma cells. The reticulum cells persisted in their hypertrophic state but the intranuclear "inclusions" disappeared, as also did the cytoplasmic bodies. There was sometimes an increase of reticular and collagenous fibres.

In one case extensive necrosis had occurred in the mediastinal nodes and in addition to the acute changes of the virus infection, free and intra-cellular toxoplasms were found.

The tonsillar epithelium contained cytoplasmic, phloxinophilic inclusion bodies especially in the early phase.

There was seldom a marked increase of blood in the spleen but free erythrocytes sometimes diffused among the lymphoid cells of the Malpighian bodies and in the same area in the early stage of distemper, a homogeneous, acellular substance was sometimes seen which failed to give a fibrin-reaction when stained by phosphotungstic acid-haematoxylin.

Tongue

The upper layers of the stratum germinativum of the glossal epidermis normally contain cytoplasmic structures which are strongly acidophilic and it was necessary to distinguish these from the distemper inclusions sometimes found in the basal layers as well as in subepithelial histiocytes and other cells. Capillary hyperaemia is commonly seen and in the later stages of the disease may be accompanied by an increase of perivascular histiocytes as well as lymphocytes and plasma cells. Occasionally bacterial infection and ulceration of the epidermis takes place with resultant polymorphonuclear infiltration.

Gastro-intestinal Tract

The organised lymphoid areas such as the solitary follicles showed reactive and degenerative changes comparable with those of the lymph nodes. In the later stages an increase in plasma cells was found especially in the lamina propria. Cytoplasmic inclusions were found in the epithelia as well as in the reticulum cells, histiocytes and sometimes the lymphocytes of the submucous tissues, being most abundant in the earlier phase.

Liver

The cytoplasm of the hepatic cells often presented a definitely foamy appearance due to numerous small droplets but frank vacuolation indicative of fatty infiltration was less marked and evidence of nuclear degeneration was not constant. Two livers showed focal necrosis of centrilobular distribution resembling that found in acute contagious hepatitis but only phloxinophilic cytoplasmic inclusion bodies were evident. Disseminated focal necrosis without a specific localisation had developed in one of the three dogs infected by toxoplasms. A frequent finding in chronic and acute cases was interstitial oedema affecting not only the interlobular stroma but also the spaces of Disse lying between the hepatic cells and their related sinusoids. Cytoplasmic inclusions were identified in hepatic cells, Kupffer cells, histiocytes and bile duct epithelium. Centrilobular congestion of various degrees was almost a constant finding but may have been terminal in some cases.

Pancreas

The parenchymal cells of the acini and islets sometimes showed regressive changes. Inclusions developed in the cytoplasm of the exocrine cells, duct epithelium and histiocytes but were not found in the endocrine cells of the islets of Langerhans. Infrequently an increase of lymphocytes, histiocytes and plasma cells had taken place in the pancreatic interstitium.

Urinary System

The cytoplasm of the renal tubular epithelium commonly showed swelling, granularity and vacuolation indicative of degeneration which in some cases would have been exaggerated by *post-mortem* change. Rarely, microscopic foci of interstitial inflammation were sparsely distributed in the cortex which may have resulted from mild leptospiral infection but chronic foci were seldom seen. One animal displayed early pyaemic nephritis as a sequel to secondary streptococcal pneumonia. The peripelvic connective tissues sometimes contained foci of reticulum cells with lymphocytic hyperplasia. Inclusions were seen at the acute phase of the infection especially in the cytoplasm of the pelvic

epithelium and in smaller numbers in the epithelium of the medulla and cortex and in histiocytes.

Interstitial hyperplasia of histiocytes and lymphocytes, epithelial degeneration and cytoplasmic inclusion bodies could be identified in the urinary bladder in most cases.

Pads

Thirty-seven dogs (74 per cent.) showed some degree of abnormality in the pads. The histological appearances were regarded as inconclusive in five animals, and as negative in eight (which is attributable in part to the resolution of hyperkeratosis in certain animals before they came to autopsy). Of the negative cases, seven showed general histological changes suggesting a late stage in the disease and one was probably an early case.

Epidermal changes varied quite widely. Those of severest kind occurred during the acute phase of distemper and appeared to subside later, about six weeks from the onset of infection as indicated by a comparative study of cases in the survey. Excessive proliferation of the basal cells in the epidermis produced irregularity of the epidermal papillae and a broadening of the cellular layers. The cells of the outer layers of the stratum malpighii and those of the stratum granulosum became swollen and exceedingly pale and in many cases their cytoplasmic boundaries could no longer be traced. Cytoplasmic vacuolation led to compression of the cell nuclei. When the upper limit of the stratum granulosum was indistinct the cellular layers merged with the keratin and, in the severer cases, the cell nuclei were seen to persist deeply into the corneum. As a result of these changes, keratinisation was improperly carried out (parakeratosis). When the active stage of proliferation and the intensified degenerative changes resolved the epidermal lamination of the pads returned to normal, although the stratum corneum tended to remain rather thick. Capillary hyperaemia often occurred in the corium and around the vessels an increase of histiocytes and lymphocytes sometimes developed. In the few cases showing bacterial infection of the pads there was polymorphonuclear infiltration of the epidermis.

Cytoplasmic inclusions in the epidermis had to be differentiated from keratohyalin which they resembled by their acidophilic staining reaction. Their presence was only accepted when they occurred in the deeper cells of the malpighian layer as round, ovoid or slightly crescentic structures surrounded by a pale cytoplasmic "halo." They were found also in sweat gland epithelium and histiocytes. Keratohyalin was most abundant in those layers showing degenerative changes where it occurred as clumps of highly pleomorphic, acidophilic material increasing in concentration as they approached the keratin itself.

Central Nervous System

Degrees of inflammation were found in the brain in all but one of the 50 dogs examined. (The negative case, which had been ill for two months, showed visceral changes suggestive of a late stage of distemper although inclusion bodies were not found. Only four blocks of brain tissue from this case were examined.)

The earliest changes were most prominent in the tissues adjacent to the ventricular system of the brain, in the pia-arachnoid and sub-pial glial mat of the brain stem, and consisted mainly of reactive changes by microglia and astrocytes. Lesions occurred focally or diffusely and were specially marked in the walls of the third and fourth ventricles, in those situations where pial invaginations came in direct contact with the cerebellar white matter, and in the white matter of the cerebellar folia. Acute swelling of oligodendrocytes usually accompanied the other glial changes. Phloxinophilic cytoplasmic inclusion bodies were found in the glia, ependymal cells, fixed and free meningeal cells and occasionally in neurones. Astrocytes sometimes showed margination of their nuclear chromatin and homogeneous intra-nuclear structures which stained a dull lavender colour in haemalum-phloxin-tartrazine sections. Progression to the stage of demyelination often occurred especially in the deep and superficial parts of the cerebellar white matter and in areas of the brain where highly myelinated tissue was closely related to the meninges, *e.g.*, near the deep pial invaginations in the ventral aspect of the cerebellum and the points of insertion of the cranial nerves. Myelinated fibres of the deeper cerebrum, thalamus and mid-brain were less frequently affected. Other glial forms such as compound corpuscles, amoeboid, swollen and multinucleated astrocytes were seen in increasing numbers as the myelin became injured.

Some axons were able to persist in the absence of myelin but distortion and fragmentation of axons could often be found. Gliosis was found in many other parts of the brain but the lesions seldom attained the same degree of severity in the earlier phase.

The onset of repair was indicated by the disappearance of actively phagocytic microglia but higher concentration of other microglial forms sometimes persisted along with an increased population of astrocytes. Swollen oligodendrocytes diminished in numbers; most residual lesions were devoid of them.

Especially in the later stages of the disease reactive changes were prominent in and around the blood vascular system of brain and cord. Such developments were notable in about 25 per cent. of the cases studied and affected grey matter as well as the white substance and meninges. The cerebral cortex was a common site. Swelling of vascular endothelia occurred so that the capillaries became unusually prominent, but there was no capillary budding although mitoses were occasionally seen in the endothelial cells. Proliferation of the perivascular (adventitial) cells took place and numbers of haemic cells contributed to the new perivascular infiltrates; polymorphonuclears remained uncommon. Neuroglia (especially the microglia and astrocytes) situated near the altered vessels showed acute reactive and sometimes degenerative changes. Adjacent neurones exhibited regressive cytoplasmic and nuclear alterations and in extreme cases they were necrosed leaving glial foci in their stead. There was continued perivascular infiltration of lymphocytes supported in some instances by plasma cells and larger mononuclears, until "cuffing" became the dominant histological feature. The degree of vascular compression and occlusion was often severe; it was sometimes associated with necrosis which may have been ischaemic. When the white matter was involved demyelination of an extreme kind resulted whereupon invasion of the lesions by numerous compound granular corpuscles, amoeboid astrocytes and

haemic cells took place. Cytoplasmic inclusions and the intra-nuclear bodies of astrocytes were found much less frequently in the later vascular and perivascular lesions.

The spinal cord usually showed only slight activation of glia in the earlier stages of distemper but distinct gliosis, vascular and perivascular reactions were seen in the secondary encephalomyelitic phase in nine cases. The most advanced cord lesions showed necrosis with demyelination.

A few groups of toxoplasms including pseudocysts were found in the brain in two cases concurrent with evidence of distemper. The parasitic focus occurred in the frontal cortex in one animal and in the mid-brain of the other.

Inclusion Bodies

Since the discovery of distemper inclusion bodies by Lentz in 1907 these structures have been studied by many workers, particularly Standfuss (1908), Sinigaglia (1913), Babes & Starcovici (1912), Sanfelice (1915), Green & Evans (1939), Watson & Plummer (1942), Sjolte (1947), Innes (1949a & c) and Lindgren (1951). Other authors, Frauchiger & Walthard (1935), Fankhauser (1951) and Potel (1951), failed to demonstrate them in their cases. In the present survey inclusions were found in 44 out of 50 cases (88 per cent.). Table IV shows their incidence in the principal tissues examined. Five of the negative cases had been ill for six weeks or more while the sixth, though said to have been ill for two or three weeks, showed microscopical changes which suggested a duration of infection comparable with the others. Although inclusions may persist for six weeks or even longer, especially in the lung and central nervous system, their numbers decrease considerably after that time. Variation was found in the incidence of the cytoplasmic bodies in relation to the various tissues examined but they were most abundant and widespread in the viscera in the earlier phase of clinical disease. The principal sites of formation observed were the tonsil, lung, lymph nodes, pancreatic exocrine cells, epithelium of the renal pelvis and urinary bladder, pads, and the microglia, astrocytes oligodendroglia and ependyma of the brain. Musculature was seldom affected. One of the few tissues apparently immune to the development of cytoplasmic inclusions was that of the pancreatic islets of Langerhans.

Nuclear inclusions have been described by a number of authors, Marinesco *et al.* (1933), Hurst *et al.* (1943), McIntyre *et al.* (1948) and McGovern *et al.* (1950). The occurrence of intranuclear inclusion bodies in canine contagious hepatitis (C.C.H.) has in the past led to some confusion between the two virus infections (de Monbreun, (1937) and Innes, (1949a)), but whereas the inclusions of C.C.H. give a positive reaction to the Feulgen staining technique for desoxyribonucleic acid, the structures encountered in distemper are Feulgen-negative. Furthermore, the inclusions of C.C.H. are widely distributed in endothelium, macrophages and hepatic cells unlike the nuclear bodies of distemper which have only been found in the reticulum cells of lymph nodes, spleen, tonsil and other reticulo-endothelial centres and in astrocytes. In the present series of dogs, nuclear inclusions, which stained weakly basophilic in haemalum-phloxin-tartrazine sections (of corrosive-formal fixed tissue) were found in those cells during acute phases of reaction. Mitotic division does not seem to occur in reticulum cells or astrocytes which develop intranuclear inclusions but whether the structures are related purely to nuclear change, rather than to viral activity, or to both, remains obscure.

TABLE IV

INCIDENCE OF CYTOPLASMIC INCLUSION BODIES IN THE PRINCIPAL TISSUES EXAMINED

Tissue	Number of cases examined	Cases with cytoplasmic inclusions	Per cent.
Lung	50	39	78
Mediastinal node ...	39	18	46
Pancreas : Exocrine cells	22	11	50
Duodenum	21	3	14
Spleen	31	12	39
Kidney	46	24	52
Bladder	36	19	53
Pads	50	20	40
Central nervous system...	50	34	68

DISCUSSION

(*a*) Pathology

Relationship of Lesions to Duration of Illness

During the present investigation an attempt was made to relate the gross and microscopical lesions as closely as possible to the length of illness in the individual animal. From a comparison of cases it appeared that certain histological features developed in a characteristic sequence. It thus seems possible, through a histological examination, to make an approximate judgment of the duration of illness up to a period of about six weeks. A reservation must still be made in respect of dogs in the first week or two of infection as experimental cases would be required to provide information about microscopical changes in the initial phase. The clinical history of dogs in this survey usually seemed a reliable index of the duration of illness but from a comparative histological study it is not unlikely that some of the dogs (about 25 per cent.) may have been ill for a longer time, even after allowance was made for the incubation period.

The Reticulo-endothelial and Lymphoid Tissues

Hypertrophy of reticulum cells together with hypertrophy and proliferation of macrophages are among the earliest tissue changes. Microglia, especially in parts of the cerebellum and brain stem, are similarly affected. These reactions may be a direct response to the presence of virus. When the need for phagocytosis of tissue debris has developed in the more advanced stages of distemper, free mobile macrophages are often numerous and may still be found even after six weeks of illness. The alveolar macrophages of the lung and the microglia of the central nervous system are examples of the continued reactive mechanism.

No final explanation can yet be offered for the extreme loss of lymphocytes in the early phases of distemper which has previously been observed by Potel (1951) and Scheitlin *et al.* (1951). Acute disintegration of these cells takes place throughout the body and is

seen most strikingly in the lymph nodes. Cytoplasmic and nuclear distortion in circulating lymphocytes may be seen concurrently in blood films. The changes found in the lymphoid system have some resemblance to the lymphocytolytic effect of anterior pituitary and adrenal cortical hormones described by Dougherty and his co-workers (1944, 1945).

Hyperplastic Epithelial Changes

The epithelial proliferation which takes place in pads and in the lungs in the acute phase suggests that distemper virus may exert a cytokinetic influence but caution must still be expressed in the interpretation of tissue changes in distemper until adequate experimental studies are made. That the pad changes may be non-specific was the view of Fankhauser (1951) who described such lesions in dogs with toxoplasmosis, but the present authors believe that concurrent distemper infection must not be excluded from a consideration of the Swiss author's cases. Pulmonary alveolar " epithelialisation " has been described in a variety of diseases of man and animals, not all of them of known infectious origin, so that the stimulating effects of virus upon those cells which are found lining the air-sacs in distemper is still a matter of conjecture. A more detailed study of the effect of distemper on the respiratory system is being made by one of the present authors (A.M.W.) and will be reported in a separate paper. The disorganisation of the pads does not reach the same intensity in every case, a fact which clinical examination will confirm. Histological examination indicates that the degree of pulmonary " epithelialisation " also varies from case to case. If, indeed, the virus plays a direct part in these hyperplastic changes it is yet uncertain whether the variation in degree is due to extrinsic factors, *e.g.*, varying epitheliotropism on the part of the virus or to intrinsic influences such as the difference in individual host susceptibility. Alveolar " epithelialisation " is part of a pattern of changes in the lung which evolves irrespective of the presence of bacterial infection.

Pulmonary Oedema

Pulmonary oedema was alluded to by McIntyre *et al.* (1948) and appears to be a constant development about two weeks after infection which sometimes persists for as long as three months. Flooding of the surfaces involved in gaseous exchange in the lung must be regarded as a major cause of respiratory distress and symptoms but its pathogenesis still remains to be elucidated. McIntyre *et al.* suggested that it might be of neurogenic origin.

Pneumonia

One of the outstanding features of distemper is pneumonia. A cellular reaction constantly occurs in the lungs and, as in many other pneumonias of viral origin, is to a great extent interstitial in site, McIntyre *et al.* (1948) and Bienfet (1953). Traces of inflammation are detectable for some weeks following the acute stage of distemper even after the disappearance of alveolar " epithelialisation " and although slight, they are considered to be of retrospective value in histological diagnosis.

In the past great importance has been placed on secondary bacterial infection in distemper, mainly in connection with lung lesions. Only 10 cases (20 per cent.) in the present series showed convincing evidence of bacterial damage in the lungs in the form of polymorphonuclear infiltration associated with organisms, and signs of *pre-mortem* bacterial invasion were encountered much less frequently in other tissues. While this is so, it would be unwise to minimise the potential effects of secondary infection by bacteria and the use of antibiotics still has its place in the treatment of distemper.*

Encephalitis

This was encountered in all but one of the dogs examined and has been followed through its developmental stages. A detailed pathological report will be presented in a separate paper (Campbell, in preparation). Despite many previous studies of changes in the central nervous system in distemper, some variation in opinion as to their nature still obtains in recent literature. From the present investigation the processes would appear to follow a fairly constant distribution up to about the fifth or sixth week of illness. The severity of the changes may, however, vary. There is evidence that certain later manifestations of encephalitis or encephalomyelitis which may develop do not result directly from the virus infection. Their exact significance has yet to be interpreted but it has been suggested by Hurst *et al.* (1943), Hurst (1952) and Trevan (1953) that there may be an underlying allergic mechanism.

Inclusion Bodies

The use of Lendrum's haemalum-phloxin-tartrazine method has been invaluable in the study and appreciation of the disease process in relation to the cytoplasmic inclusion bodies which appear to be pathognomonic for distemper within certain time limits. Failure on the part of previous authors to demonstrate inclusions was probably due to two factors. Firstly, their use of some form of haematoxylin-eosin, many varieties of which have been employed experimentally on material from the present series of cases without giving sufficient differentiation between cellular cytoplasm and inclusion bodies to permit the latter to be easily recognised. The careful use of haemalum and phloxin with cellosolve as differentiator will result in crimson inclusions standing out clearly against a pale blue cytoplasmic background. The inclusions may then be readily found even when present in very small numbers as in the late stages of pneumonia. A second factor which precluded the recognition of inclusions has been the tendency of some authors to study animals in the later stages of disease when the structures are scanty or even absent. From the work of Watson & Plummer (1942) on experi-

* Aerobic cultures on blood agar were made from the lungs of 70 cases of distemper, including most of the dogs in the present survey : 63 per cent. of cultures proved sterile ; 22 per cent. yielded streptococci ; 13 per cent. *Bact. coli* ; 8 per cent. *P. vulgaris* and 4 per cent. *Staph. albus*. *P. pyocyanea* was isolated from one dog, and *Salm. typhi-murium* from a puppy involved in a kennel outbreak. *H. bronchisepticus* was not obtained from any dog in the series, a finding which confirms the observation of McIntyre *et al.* (1948) that the organism now appears to be an uncommon pathogen of dogs despite its time-honoured reputation as a secondary invader in distemper.

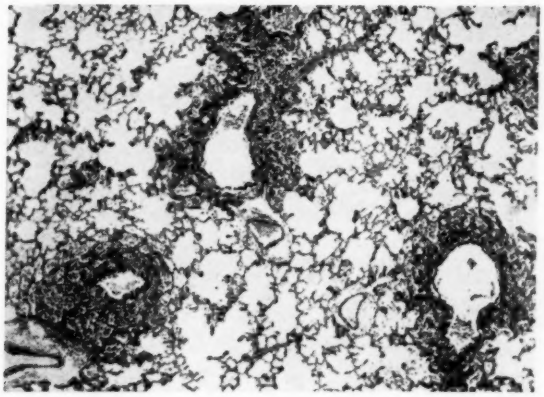

FIG. 8.—Lung : Early pneumonia ; showing cellular reaction in and around bronchioles and their related alveoli. There is slight alveolar and interstitial oedema. Haemalum-eosin-azure. × 32.

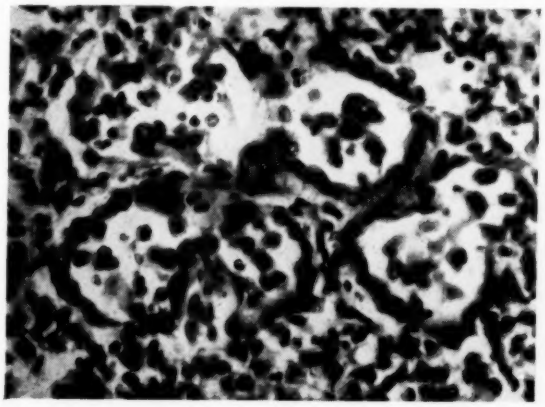

FIG. 9.—Lung : Several alveoli show a distinct lining of flattened cells. There is a mononuclear reaction, and polymorphonuclears can also be seen in this case. Haemalum-eosin-azure. × 410.

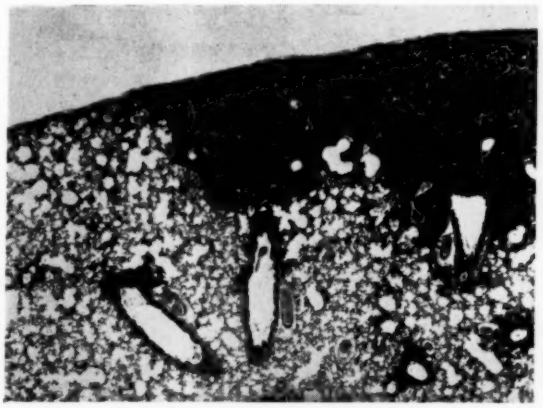

FIG. 10.—Lung : Showing cellular changes diffusing in the subpleural area. Haemalum-eosin-azure. × 13.

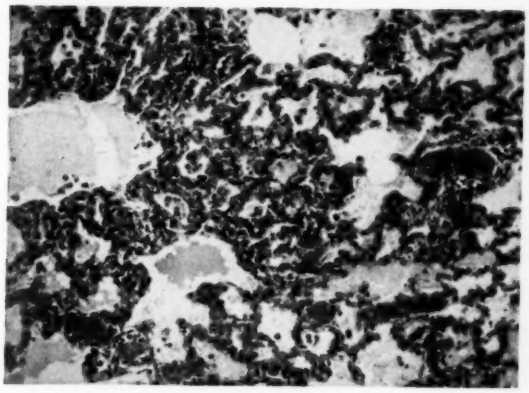

FIG. 11. Lung : The residual pneumonia of late distemper. There is slight bronchiolar and alveolar catarrh, but no evidence remains of " epithelialisation " as shown in Fig. 10. The interstitium of venules shows lymphocytic infiltration. There is persistent oedema. Haemalum-eosin. × 110.

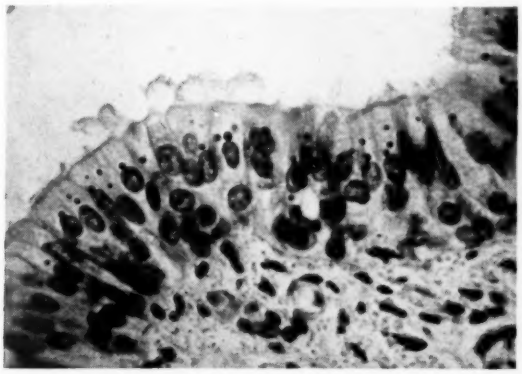

FIG. 12. Bronchial mucosa. Many epithelial cells contain round or oval cytoplasmic inclusion bodies. Haemalum-phloxin-tartrazine (Green filter). × 530.

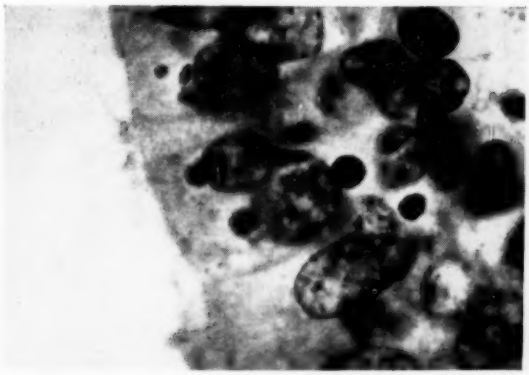

FIG. 13.—Tracheal epithelium : Showing several inclusions surrounded by cytoplasmic " halos." Two inclusions have an internal particulate structure. Haemalum-phloxin-tartrazine (Green filter). × 1,600.

mentally infected ferrets, cytoplasmic inclusions develop about 10 days after infection. The present survey indicates that they persist until about the fifth or sixth week, thereafter diminishing rapidly in numbers in certain tissues, *e.g.*, lymph nodes, urinary tract, but rather less so in the lungs and brain.

The nuclear " inclusion bodies " found during phases of acute reaction in reticulum cells and astrocytes are more limited in tissue distribution than the cytoplasmic inclusions. Although giving an eosinophilic reaction like the cytoplasmic bodies in haematoxylin-eosin preparations, the nuclear structures differ further in sections stained by the haemalum-phloxin-tartrazine method. It is therefore considered that the cytoplasmic inclusions have greater diagnostic value in distemper and that they are more likely to represent a phase in the development of the virus itself.

Toxoplasmosis

The discovery of toxoplasma infection in 3 out of 50 cases (6 per cent.) of distemper must be specially noted. Further observations on the relationship between the two diseases will be given in a separate paper.

The disease as a whole offers a rich field for investigation into the reaction of tissues to a pantropic virus.

(*b*) Clinical

It is appreciated that some dogs may suffer from distemper without showing such severe signs as those described, *e.g.*, mild cases in a kennel outbreak might reasonably be considered as distemper when in-contacts on the premises show diagnostic clinical and *post-mortem* signs. However, such a mild degree of infection in an isolated case would not be associated with symptoms sufficiently marked to be diagnostic. It is also appreciated that other severe symptoms may occur which do not conform with the general criteria for inclusion in the series but these are in the minority of the sporadic cases presented at a clinic, although they may be commoner in some outbreaks in breeders' kennels which do not form any part of our material.

Many previous articles do not mention clinical signs in detail and often it appears to be assumed that they are so well known as not to merit description.

In contrast, much has been written on aspects of the clinical signs of illness variously described as " so-called " " atypical " and " nervous " distemper, but in the review of the literature little or no reference was made to these articles, as our interest is on the syndrome which is more commonly encountered.

As long ago as 1809 Jenner described clinical symptoms in distemper which had fewer points of difference in comparison with our present findings than those of some later writers. Carré (1905), dealing with experimental dogs, did not mention sufficient clinical details for a similar comparison to be made. Dunkin & Laidlaw (1926) described a relatively mild disease in experimental dogs, and while recording that only a very small proportion showed severe respiratory involvement, they mentioned that this was not in agreement with findings in the field at that time. Nervous signs were noted in only 10 per cent. of their cases, although this may possibly be explained by the fact that no dog was allowed to live much longer than three to four weeks. The di-phasic temperature curve described by the same workers is a feature which is difficult to record in sporadic cases in the field.

The work by McIntyre *et al.* (1948) was stimulating because it focused attention on a syndrome having features in common with distemper yet believed by the authors to be a distinct entity which they named " hard pad " disease. This belief was based largely on histological evidence, but partly on clinical grounds which we do not think they made sufficiently clear. From the evidence which we have accumulated we are unable to differentiate on such points as age-incidence, character of oculo-nasal discharge, and presence or absence of hyperkeratosis of the foot-pads, a syndrome which stands clearly apart from previous descriptions of distemper, and we are of the opinion that at present we recognise only one disease entity.

In 1951, Scheitlin (who previously had drawn the attention of McIntyre *et al.* to the feature of hardening of the foot-pads) in collaboration with her co-workers, described a syndrome which on clinical grounds would be impossible to differentiate clearly from that described by us, and while stating that the condition is immunobiologically distinct from distemper, the authors gave no data to support this conclusion. It appears quite possible that hardening of the pads was a characteristic of some cases of distemper long before attention was drawn to the fact (Innes, 1949b ; Fankhauser, 1951), and Pugh (1948) was of the opinion that he had previously observed the lesion.

Furumoto's clinical observations on dogs kept in hospital are similar to those of the present writers. It seems likely, therefore, that distemper in this country many years ago and at the present time is similar to the contemporary disease in Europe and the United States.

(*c*) General

The present work was designed as an integrated study of distemper as it occurs in an urban area, cases being selected during a period of almost two years. Diagnosis has been based on clinical observations and confirmed by pathological studies, while the data obtained was used in an attempt to analyse the pattern of infection and tissue reaction. The differential diagnosis of distemper may be difficult but by the application of the clinical criteria stated in an earlier part of this paper the accuracy of diagnosis appears to have been high when all canine out-patients seen in the Glasgow clinics and later taken to *post-mortem* are considered. The exactness of diagnosis in all clinical cases is still unknown. Diagnosis is most difficult in the earlier stages of infection. Thereafter the clinical signs give increasing evidence of distemper and such nervous complications as may arise, notably epileptiform crises and myoclonic spasms (chorea) are of considerable if belated value in diagnosis.

The duration of illness appeared to correspond in most cases with the stage of development of the lesions but observations on a small number of dogs (some excluded from the survey because of the lack of cardinal symptoms) indicated that the lesions present were older than the case histories suggested. Discrepancy between clinical and pathological findings is not a new experience having been commented upon by previous workers

(Frauchiger & Walthard, 1935). Histological examination gives reason to believe that tissue changes such as pneumonia may exist subclinically though perhaps not always to the same degree as in the more typical case. This would afford an explanation for the dog which develops "nervous distemper," sometimes quite suddenly, unheralded by catarrhal or other earlier signs.

Neuropathological studies have suggested that some degree of inflammation occurs in the brain in a greater proportion of distemper cases than has hitherto been suspected. After about the third week of infection encephalitis has been found to be an almost constant development. Such a view has not always been held in the past. The work of Dunkin & Laidlaw (1926) is sometimes taken to show that true inflammatory changes do not always affect the central nervous system in "classical" distemper. This, we submit, cannot be accepted without question. Dogs used by these workers were mainly studied during the acute stages of the disease, before inflammatory lesions in the brain may reach their peak. Furthermore, the regions of the brain studied by Dunkin & Laidlaw were not stated and it is possible that earlier encephalitic lesions were undetected, as they are usually concentrated in the brain-stem and cerebellum. "Encephalitis" in relation to distemper does not imply inflammation throughout the brain. Nor is the lack of clinical nervous signs proof of the absence of encephalitis. Three cases of distemper in our series showed severe inflammatory and demyelinating lesions, mainly in the cerebellum, but did not display any nervous disability except weakness. Other, earlier, encephalitic cases were without hysterical, epileptiform, ataxic, choreiform or paraplegic signs and did not show any other aberration of behaviour.

The question may be asked whether encephalitis occurs in the dog apart from distemper, excluding well-defined forms of disease (*e.g.*, rabies). The present authors agree that cases of encephalitis are encountered without previous clinical signs of distemper having been detected in animals which fail to show conclusive visceral evidence of distemper grossly or microscopically. In such animals cytoplasmic inclusion bodies are not demonstrable in the thoracic and abdominal organs or in the central nervous system and attempts to transmit an infectious agent to the dog or ferret may be negative (McIntyre *et al.*, 1948). Most of these animals show a disseminated encephalomyelitis characterised largely by perivascular lymphocytic infiltration. The age of the affected dog is usually over one year, so that a term sometimes applied was "old dog encephalitis." McIntyre *et al.* included such cases in Group III of their classification of canine encephalitis, but Trevan in a later paper (1953) suggested that they may represent a chronic phase of distemper.

As yet it is not possible to prove a relationship between distemper and this type of illness but lesions resembling those of the disseminated encephalomyelitis which were encountered in the present series occurred in the great majority of cases in the dog with the longer duration of illness. This fact, together with the decline of the visceral lesions and the disappearance of inclusion bodies in the later stages of acute distemper suggests that the condition may not be unconnected. The reason for the continued chronic encephalitis remains to be explained although Hurst (1952) and Trevan (1953) suggested that it may have an allergic basis.

The pulmonary changes in distemper must be regarded as of considerable importance, not least from the clinical viewpoint. Pneumonia was a constant finding in the survey dogs and the cytology and anatomical (*i.e.*, interstitial) distribution of lesions point to activity of the virus itself, with secondary bacterial infection playing not only a minor rôle, but also an inconstant one. Inflammation in the lung may or may not cause clinically appreciable dyspnoea. Pulmonary oedema, the cause of which is still uncertain, is also a prominent part of the distemper disease process and is probably responsible for much of the respiratory difficulty.

Abnormalities were detected microscopically in the pads of most of the dogs in this series, although they were not always observed clinically. To some extent the stage of illness appeared to govern the degree of disorganisation in that affected pads softened and showed evidence of return to their normal architecture in dogs with longer clinical records. It is possible that other factors can influence the degree of change in the epidermis of the pads. McIntyre *et al.* (1948) failed to produce hyperkaratosis in a number of dogs infected with a "hard pad" strain of virus, but they claimed to have caused histologically detectable thickening of the pads in three animals of a group which was given a sulphonamide orally, in addition to the virus. As sulphonamides were seldom used in our series, these drugs cannot be entirely blamed for the epidermal changes. Whether or not hyperkeratosis is a recently acquired lesion in distemper infection has been a matter for much debate and it does not seem possible to give a definite answer to the question. Pugh (1948), opening the discussion to the paper of McIntyre *et al.*, stated his conviction that he had "seen this 'hard pad' condition before but never in quite such a well-marked form." Innes (1949b) believed that hyperkeratosis could have been overlooked in the past and Fankhauser (1951) stated that it was present in Switzerland before the recent war. The Swiss author also considered hard pads to be a non-specific change as he had seen them in dogs suffering from toxoplasmosis. In our experience toxoplasmosis has always been encountered in the dog together with histological evidence of concurrent distemper. This point will be dealt with in a separate paper.

Only careful experimental work on many dogs can elucidate accurately the factors responsible for the clinical and pathological variations which undoubtedly occur in distemper. The disease is by no means unique in its diversity as many other infections vary greatly in their forms. The reasons for differences in individual reactions to infection are little understood. Innate resistance, including breed resistance (Hardenbergh, 1923; Rubarth, 1947); the plane of nutrition (McIntyre *et al.*, 1948); the concurrent effect of endogenous or exogenous poisons (Verlinde, 1948) have each been suggested as possibly influencing the balance between host and parasite. Concurrent, progressive, bacterial infection no doubt alters the clinical signs in some cases but its influence has almost certainly been over-emphasised in the past. The significance of toxoplasmosis as a complication of distemper has yet

to be fully assessed. The virulence of viral strains is undoubtedly of importance (Mansi, 1951 ; Larin, 1954) and it can be altered as shown by the decrease in pathogenicity for dogs of distemper virus transmitted in serial passage through ferrets (Green, 1942) or through the chorio-allantoic membranes of developing chicks (Cabasso & Cox, 1952). Circulation of virus through a canine population, especially in cities, may be comparatively rapid and is possibly accompanied by variation in the virulence of the organism. An illustration of the potential power of distemper virus in the dog and ferret was given by the studies of Mansi (1951) on a field strain, Glasgow S123, but even this potent strain produced "typical" catarrhal distemper in ferrets again after five parenteral passages through that species (Ministry of Agriculture and Fisheries Report on Animal Health Services, 1953). The proposal by Larin (1954) and Larin & Hodgman (1954) that strains of virus may be placed into three groups on a basis of clinical and immunological studies in ferrets is a stimulating one and technical details of this work are awaited with interest.

Because of its clinical variations there have been many attempts to subclassify distemper according to the symptoms shown. The existence of separate disease entities has even been suggested in cases where probably distemper infection alone was present. In contrast is the inclusion by Whitney & Whitney (1953) of diseases of different aetiology (*e.g.*, contagious hepatitis, leptospirosis) within the term "distemper complex" on the grounds that they possess certain features in common with distemper. Such a practice would not however lead to a better understanding of these conditions.

The combined evidence of clinical and pathological examinations on the 50 dogs of this series points to the unity of the distemper disease process. Scrutiny of many additional cases outwith the present series (over 200 have been examined pathologically) in no way weakens this opinion but, on the contrary, gives to it considerable support. It is recommended that only the term "distemper" should be applied to the disease described.

Summary

1. A report is given of the clinical and pathological findings in 50 cases of naturally acquired distemper.

2. Diagnosis was initially based on clinical examination alone and appears to have been upheld by the pathological findings in every case.

3. In addition to an analysis of the major signs shown by the 50 dogs a general clinical description of distemper is given.

4. The principal microscopic changes were found in the reticulo-endothelial lymphoid, respiratory and central nervous systems. Hyperplasia of the epidermis of the pads was also a notable feature.

5. Cytoplasmic inclusion bodies were found in a wide variety of cells and are regarded as pathognomonic for distemper. It is emphasised that they may not be detected in the early and late phases of the disease.

6. The clinical and pathological data obtained indicate a single disease entity which, it is recommended, should simply be called distemper.

Acknowledgments.—We wish to express our thanks to Professor W. L. Weipers and Professor J. W. Emslie for their guidance in the preparation of this paper, to Mr. S. W. Michna for his valuable co-operation, and to all other colleagues in the Pathology and Clinical Departments who offered helpful criticism. It is with pleasure also that we express indebtedness to Mr. W. Penny, F.I.M.L.T., and Mr. W. McLennan, A.I.M.L.T., for technical assistance ; to Mrs. E. L. Leighton for considerable work of translation ; and to Miss J. Roberts for her examination of clinical records. The photographs were prepared by Mr. A. Finnie, of the University Veterinary School.

References

BABES, V., & STARCOVICI, C. (1912). *Comp. Rend. Soc. Biol.* **2.** 229.
BIENFET, V. (1953). *Ann. Med. vet.* **97.** 51.
BLAINE, D. (1817). "Canine Pathology." London.
BURKHART, R. L., POPPENSIEK, G. C., & ZINK, A. (1950). *Vet. Med.* **45.** 157.
CABASSO, V. J. (1952). *Ibid.* **47.** 417.
——— & COX, H. R. (1952). *Cornell Vet.* **42.** 96.
CARRE, H. (1905). *Comp. Rend. Acad. Sci.* **140.** 689 & 1489.
COHRS, P. (1951). *Dtsch. tierärtztl. Wochr.* **58.** 129.
DE MONBREUN, W. A. (1937). *Amer. J. Path.* **13.** 187.
DOUGHERTY, T. F., & WHITE, A. (1945). *Amer. J. Anat.* **77.** 81.
———, CHASE, J. H., & WHITE, A. (1944). *Proc. Soc. exp. Biol., N.Y.* **57.** 295.
———, ——— & ———. (1945). *Ibid.* **58.** 135.
———, WHITE, A., & CHASE, J. H. (1945). *Ibid.* **59.** 172.
DUNKIN, C. W., & LAIDLAW, P. P. (1926). *J. comp. Path.* **39.** 201, 213 & 222.
FANKHAUSER, R. (1951). *Schweiz. Arch. Tierheilk.* **93.** 717 & 796.
FRAUCHIGER, E., & WALTHARD, K. M. (1935). *Arch. wiss. prakt. Tierheilk.* **69.** 231.
FURUMOTO, H. H. (1952). *N. Amer. Vet.* **33.** 550.
GORET, P. (1950). *Vet. Rec.* **62.** 6.
GREEN, R. G. (1941). *J. Amer. vet. med. Ass.* **99.** 45.
———. (1942). *N. Amer. Vet.* **23.** 526.
——— & EVANS, C. A. (1939). *Amer. J. Hyg.* **29.** 73.
HARDENBERGH, J. G. (1923). *J. Amer. vet. med. Ass.* **78.** 309.
HURST, E. W. (1952). "Multiple Sclerosis and the Demyelinating Diseases. Experimental Demyelination in relation to Human and Animal Disease." Williams & Wilkins, Baltimore.
———, COOK, B. T., & MELVIN, P. (1943). *Aust. J. exp. Biol. med. Sci.* **21.** 115.
INNES, J. R. M. (1949a). *Vet. Rec.* **61.** 73 & 173.
———. (1949b). *Vet. Med.* **44.** 421 & 467.
———. (1949c). *Br. vet. J.* **105.** 99.
JENNER, E. (1809). *Med. chir. Trans.* **1.** 263.
KOPROWSKI, H., JERVIS, G. A., JAMES, T. R., BURKHART, R. L., & POPPENSIEK, G. C. (1950). *Amer. J. Hyg.* **51.** 63.
LARIN, N. M. (1954). "Nature." 173, 174.
——— & HODGMAN, S. F. J. (1954). *Vet. Rec.* **66.** 339.
LENTZ, O. (1907). *Zbl. Bakt.* **42** (Ref. Suppl.). 126.
LINDGREN, N. O. (1951). *Nord. veterinärmed.* **3.** 403.
MANSI, W. (1951). *Br. vet. J.* **107.** 214.
MARINESCO, G., DRAGANESCO, S., & STRUESCO, G. (1933). *Ann. Inst. Past.* **51.** 215.
MCGOVERN, V. J., STEEL, J. D., WYKE, B. D., & DODSON, M. E. (1950). *Aust. J. exp. Biol. med. Sci.* **28.** 434.
MCINTYRE, A. B., TREVAN, D. J., & MONTGOMERIE, R. F. (1948). *Vet. Rec.* **60.** 635.
NIJLAND, G. J. (1952). *Tijdschr. Diergeneesk.* **77.** 511.
POTEL, K. (1951). *Exp. Veterinarmed.* **4.** 44.
PUGH, L. P. (1948). *Vet. Rec.* **60.** 639.
Report on Animal Health Services (Ministry of Agriculture and Fisheries). (1953).
RUBARTH, S. (1947). *Acta. path. microbiol. Scand.* Suppl. LXIX.

(Continued at foot of page 632)

(Frauchiger & Walthard, 1935). Histological examination gives reason to believe that tissue changes such as pneumonia may exist subclinically though perhaps not always to the same degree as in the more typical case. This would afford an explanation for the dog which develops "nervous distemper," sometimes quite suddenly, unheralded by catarrhal or other earlier signs.

Neuropathological studies have suggested that some degree of inflammation occurs in the brain in a greater proportion of distemper cases than has hitherto been suspected. After about the third week of infection encephalitis has been found to be an almost constant development. Such a view has not always been held in the past. The work of Dunkin & Laidlaw (1926) is sometimes taken to show that true inflammatory changes do not always affect the central nervous system in "classical" distemper. This, we submit, cannot be accepted without question. Dogs used by these workers were mainly studied during the acute stages of the disease, before inflammatory lesions in the brain may reach their peak. Furthermore, the regions of the brain studied by Dunkin & Laidlaw were not stated and it is possible that earlier encephalitic lesions were undetected, as they are usually concentrated in the brain-stem and cerebellum. "Encephalitis" in relation to distemper does not imply inflammation throughout the brain. Nor is the lack of clinical nervous signs proof of the absence of encephalitis. Three cases of distemper in our series showed severe inflammatory and demyelinating lesions, mainly in the cerebellum, but did not display any nervous disability except weakness. Other, earlier, encephalitic cases were without hysterical, epileptiform, ataxic, choreiform or paraplegic signs and did not show any other aberration of behaviour.

The question may be asked whether encephalitis occurs in the dog apart from distemper, excluding well-defined forms of disease (*e.g.*, rabies). The present authors agree that cases of encephalitis are encountered without previous clinical signs of distemper having been detected in animals which fail to show conclusive visceral evidence of distemper grossly or microscopically. In such animals cytoplasmic inclusion bodies are not demonstrable in the thoracic and abdominal organs or in the central nervous system and attempts to transmit an infectious agent to the dog or ferret may be negative (McIntyre *et al.*, 1948). Most of these animals show a disseminated encephalomyelitis characterised largely by perivascular lymphocytic infiltration. The age of the affected dog is usually over one year, so that a term sometimes applied was "old dog encephalitis." McIntyre *et al.* included such cases in Group III of their classification of canine encephalitis, but Trevan in a later paper (1953) suggested that they may represent a chronic phase of distemper.

As yet it is not possible to prove a relationship between distemper and this type of illness but lesions resembling those of the disseminated encephalomyelitis which were encountered in the present series occurred in the great majority of cases in the dog with the longer duration of illness. This fact, together with the decline of the visceral lesions and the disappearance of inclusion bodies in the later stages of acute distemper suggests that the condition may not be unconnected. The reason for the continued chronic encephalitis remains to be explained although Hurst (1952) and Trevan (1953) suggested that it may have an allergic basis.

The pulmonary changes in distemper must be regarded as of considerable importance, not least from the clinical viewpoint. Pneumonia was a constant finding in the survey dogs and the cytology and anatomical (*i.e.*, interstitial) distribution of lesions point to activity of the virus itself, with secondary bacterial infection playing not only a minor rôle, but also an inconstant one. Inflammation in the lung may or may not cause clinically appreciable dyspnoea. Pulmonary oedema, the cause of which is still uncertain, is also a prominent part of the distemper disease process and is probably responsible for much of the respiratory difficulty.

Abnormalities were detected microscopically in the pads of most of the dogs in this series, although they were not always observed clinically. To some extent the stage of illness appeared to govern the degree of disorganisation in that affected pads softened and showed evidence of return to their normal architecture in dogs with longer clinical records. It is possible that other factors can influence the degree of change in the epidermis of the pads. McIntyre *et al.* (1948) failed to produce hyperkaratosis in a number of dogs infected with a "hard pad" strain of virus, but they claimed to have caused histologically detectable thickening of the pads in three animals of a group which was given a sulphonamide orally, in addition to the virus. As sulphonamides were seldom used in our series, these drugs cannot be entirely blamed for the epidermal changes. Whether or not hyperkeratosis is a recently acquired lesion in distemper infection has been a matter for much debate and it does not seem possible to give a definite answer to the question. Pugh (1948), opening the discussion to the paper of McIntyre *et al.*, stated his conviction that he had "seen this 'hard pad' condition before but never in quite such a well-marked form." Innes (1949b) believed that hyperkeratosis could have been overlooked in the past and Fankhauser (1951) stated that it was present in Switzerland before the recent war. The Swiss author also considered hard pads to be a non-specific change as he had seen them in dogs suffering from toxoplasmosis. In our experience toxoplasmosis has always been encountered in the dog together with histological evidence of concurrent distemper. This point will be dealt with in a separate paper.

Only careful experimental work on many dogs can elucidate accurately the factors responsible for the clinical and pathological variations which undoubtedly occur in distemper. The disease is by no means unique in its diversity as many other infections vary greatly in their forms. The reasons for differences in individual reactions to infection are little understood. Innate resistance, including breed resistance (Hardenbergh, 1923; Rubarth, 1947); the plane of nutrition (McIntyre *et al.*, 1948); the concurrent effect of endogenous or exogenous poisons (Verlinde, 1948) have each been suggested as possibly influencing the balance between host and parasite. Concurrent, progressive, bacterial infection no doubt alters the clinical signs in some cases but its influence has almost certainly been over-emphasised in the past. The significance of toxoplasmosis as a complication of distemper has yet

to be fully assessed. The virulence of viral strains is undoubtedly of importance (Mansi, 1951 ; Larin, 1954) and it can be altered as shown by the decrease in pathogenicity for dogs of distemper virus transmitted in serial passage through ferrets (Green, 1942) or through the chorio-allantoic membranes of developing chicks (Cabasso & Cox, 1952). Circulation of virus through a canine population, especially in cities, may be comparatively rapid and is possibly accompanied by variation in the virulence of the organism. An illustration of the potential power of distemper virus in the dog and ferret was given by the studies of Mansi (1951) on a field strain, Glasgow S123, but even this potent strain produced "typical" catarrhal distemper in ferrets again after five parenteral passages through that species (Ministry of Agriculture and Fisheries Report on Animal Health Services, 1953). The proposal by Larin (1954) and Larin & Hodgman (1954) that strains of virus may be placed into three groups on a basis of clinical and immunological studies in ferrets is a stimulating one and technical details of this work are awaited with interest.

Because of its clinical variations there have been many attempts to subclassify distemper according to the symptoms shown. The existence of separate disease entities has even been suggested in cases where probably distemper infection alone was present. In contrast is the inclusion by Whitney & Whitney (1953) of diseases of different aetiology (*e.g.*, contagious hepatitis, leptospirosis) within the term "distemper complex" on the grounds that they possess certain features in common with distemper. Such a practice would not however lead to a better understanding of these conditions.

The combined evidence of clinical and pathological examinations on the 50 dogs of this series points to the unity of the distemper disease process. Scrutiny of many additional cases outwith the present series (over 200 have been examined pathologically) in no way weakens this opinion but, on the contrary, gives to it considerable support. It is recommended that only the term "distemper" should be applied to the disease described.

Summary

1. A report is given of the clinical and pathological findings in 50 cases of naturally acquired distemper.

2. Diagnosis was initially based on clinical examination alone and appears to have been upheld by the pathological findings in every case.

3. In addition to an analysis of the major signs shown by the 50 dogs a general clinical description of distemper is given.

4. The principal microscopic changes were found in the reticulo-endothelial lymphoid, respiratory and central nervous systems. Hyperplasia of the epidermis of the pads was also a notable feature.

5. Cytoplasmic inclusion bodies were found in a wide variety of cells and are regarded as pathognomonic for distemper. It is emphasised that they may not be detected in the early and late phases of the disease.

6. The clinical and pathological data obtained indicate a single disease entity which, it is recommended, should simply be called distemper.

Acknowledgments.—We wish to express our thanks to Professor W. L. Weipers and Professor J. W. Emslie for their guidance in the preparation of this paper, to Mr. S. W. Michna for his valuable co-operation, and to all other colleagues in the Pathology and Clinical Departments who offered helpful criticism. It is with pleasure also that we express indebtedness to Mr. W. Penny, F.I.M.L.T., and Mr. W. McLennan, A.I.M.L.T., for technical assistance ; to Mrs. E. L. Leighton for considerable work of translation ; and to Miss J. Roberts for her examination of clinical records. The photographs were prepared by Mr. A. Finnie, of the University Veterinary School.

References

BABES, V., & STARCOVICI, C. (1912). *Comp. Rend. Soc. Biol.* **2.** 229.
BIENFET, V. (1953). *Ann. Med. vet.* **97.** 51.
BLAINE, D. (1817). "Canine Pathology." London.
BURKHART, R. L., POPPENSIEK, G. C., & ZINK, A. (1950). *Vet. Med.* **45.** 157.
CABASSO, V. J. (1952). *Ibid.* **47.** 417.
——— & COX, H. R. (1952). *Cornell Vet.* **42.** 96.
CARRE, H. (1905). *Comp. Rend. Acad. Sci.* **140.** 689 & 1489.
COHRS, P. (1951). *Dtsch. tierärtztl. Wochr.* **58.** 129.
DE MONBREUN, W. A. (1937). *Amer. J. Path.* **13.** 187.
DOUGHERTY, T. F., & WHITE, A. (1945). *Amer. J. Anat.* **77.** 81.
———, CHASE, J. H., & WHITE, A. (1944). *Proc. Soc. exp. Biol., N.Y.* **57.** 295.
———, ——— & ———. (1945). *Ibid.* **58.** 135.
———, WHITE, A., & CHASE, J. H. (1945). *Ibid.* **59.** 172.
DUNKIN, C. W., & LAIDLAW, P. P. (1926). *J. comp. Path.* **39.** 201, 213 & 222.
FANKHAUSER, R. (1951). *Schweiz. Arch. Tierheilk.* **93.** 717 & 796.
FRAUCHIGER, E., & WALTHARD, K. M. (1935). *Arch. wiss. prakt. Tierheilk.* **69.** 231.
FURUMOTO, H. H. (1952). *N. Amer. Vet.* **33.** 550.
GORET, P. (1950). *Vet. Rec.* **62.** 6.
GREEN, R. G. (1941). *J. Amer. vet. med. Ass.* **99.** 45.
———. (1942). *N. Amer. Vet.* **23.** 526.
——— & EVANS, C. A. (1939). *Amer. J. Hyg.* **29.** 73.
HARDENBERGH, J. G. (1923). *J. Amer. vet. med. Ass.* **78.** 309.
HURST, E. W. (1952). "Multiple Sclerosis and the Demyelinating Diseases. Experimental Demyelination in relation to Human and Animal Disease." Williams & Wilkins, Baltimore.
———, COOK, B. T., & MELVIN, P. (1943). *Aust. J. exp. Biol. med. Sci.* **21.** 145.
INNES, J. R. M. (1949a). *Vet. Rec.* **61.** 73 & 173.
———. (1949b). *Vet. Med.* **44.** 421 & 467.
———. (1949c). *Br. vet. J.* **105.** 99.
JENNER, E. (1809). *Med. chir. Trans.* **1.** 263.
KOPROWSKI, H., JERVIS, G. A., JAMES, T. R., BURKHART, R. L., & POPPENSIEK, G. C. (1956). *Amer. J. Hyg.* **51.** 63.
LARIN, N. M. (1954). "Nature." 173, 174.
——— & HODGMAN, S. F. J. (1954). *Vet. Rec.* **66.** 339.
LENTZ, O. (1907). *Zbl. Bakt.* **42** (Ref. Suppl.). 126.
LINDGREN, N. O. (1951). *Nord. veterinärmed.* **3.** 403.
MANSI, W. (1951). *Br. vet. J.* **107.** 214.
MARINESCO, G., DRAGANESCO, S., & STRUESCO, G. (1933). *Ann. Inst. Past.* **51.** 215.
MCGOVERN, V. J., STEEL, J. D., WYKE, B. D., & DODSON, M. E. (1950). *Aust. J. exp. Biol. med. Sci.* **28.** 434.
MCINTYRE, A. B., TREVAN, D. J., & MONTGOMERIE, R. F. (1948). *Vet. Rec.* **60.** 635.
NIJLAND, G. J. (1952). *Tijdschr. Diergeneesk.* **77.** 511.
POTEL, K. (1951). *Exp. Veterinarmed.* **4.** 44.
PUGH, L. P. (1948). *Vet. Rec.* **60.** 639.
Report on Animal Health Services (Ministry of Agriculture and Fisheries). (1953).
RUBARTH, S. (1947). *Acta. path. microbiol. Scand.* Suppl. LXIX.

(Continued at foot of page 632)

A Contribution to the Early Embryology of the Sheep.

An Attempt to Correlate the Developmental Stage of the Descending Ovum with the Place of Recovery.

BY

R. HADEK

University of Glasgow Veterinary School

Introduction and Literature

THE object of this work was to gather accurate information on the features of the cleaving and non-segmenting ova of the sheep at different segments of the oviduct.

While Bear (1828) has established that ovulation in mammals is spontaneous, Hausmann (1840), Bischoff (1842) and Assheton (1898) were the first to recover ova from the ewe's oviduct. More recent papers on the ovum of the sheep were published by Allen & McKenzie (1931), Allen *et al.* (1931), Clark (1934), Cole & Miller (1932), Green & Winters (1935), Kelley (1937) McKenzie & Allen (1935) and McKenzie & Terril (1935 and 1937).

In the majority of these papers the developmental state (age) of the cleaving egg is either correlated (i) to the time which had elapsed from the beginning of oestrus (Allen & McKenzie, 1931, Allen *et al.* 1931, McKenzie & Allen, 1933, and McKenzie & Terril, 1935 & 1937), or (ii) to the duration of time spent since mating has taken place (Clark, 1934, Cole & Miller, 1932, Green & Winters, 1935). In one paper only is (iii) the place of recovery correlated to the period of time since the cessation of heat (Kelley, 1937).

The Breeding Season and the Sexual Cycle in the Ewe

The domestic sheep in Scotland is a seasonally polyoestric animal. The breeding season starts in autumn, but its beginning is influenced by breed, by geographical and by thermal influences. Some of the lowland sheep breed for considerably longer periods than those of the highlands. The majority of sheep used in the present work were blackfaced highland sheep and their sexual season begins in late October. The average length of the cycle is 16.5 days (Grant, 1934). Oestrus or heat averages 20 to 24 hours; met-oestrus two days; early luteal phase, some two to three days; di-oestrus or late luteal phase, some seven to eight days; and finally, if the sheep is not successfully mated, pro-oestrus, two to three days.

Opinion as to the time of ovulation is varied. While some authors consider that ovulation takes place in the later hours of oestrus (Clark, 1934), others claim that it occurs after cessation of heat) (Grant, 1934, McKenzie & Terril, 1937). The average number of ova shed at individual ovulation by the Blackface is 1.87 (Grant, 1934).

Material and Methods

The 81 sheep ova described in this paper were obtained from the genital tracts of 60 sheep which were collected from the Glasgow abattoir immediately after slaughter. Nothing was known of the previous history of the animals and the stage of the sexual cycle was therefore judged by luteal development.

If the appearance of the corpus luteum suggested fairly recent ovulation, the whole genital tract was placed in a container and kept at a temperature of about 30° C. until return to the laboratory half an hour later for detailed examination. In the laboratory the suspensory ligaments were removed and the oviducts cut into three equal parts. Each part and the uterus then washed out by the method of Heuser & Streeter (1927).

Description of Ova

Ova Recovered from the Oviduct

Six ova were recovered from the ovarian third of the oviduct. All of them were in the one-cell stage and they could be divided into two groups depending on whether a perivitelline space was present or absent.

Group A included three eggs which did not show any perivitelline space since the ovum completely filled the zona pellucida. In one ovum a number of spermatozoa were attached to the zona pellucida but did not seem to have penetrated it (Fig. 1). The zona pellucida although apparently structurally homogeneous, showed darker and lighter areas, and some cell debris was attached to it. The vitelline globules were small and evenly dispersed.

Group B contained three eggs which were similar to those in Group A except that they did not completely fill the zona pellucida but showed a small semilunar perivitelline space (Fig. 2).

Twenty-three eggs were recovered in the middle third of the oviduct. Of them 17 were in the one-cell stage and six had undergone cleavage.

The 17 undivided eggs were identical in appearance except for the size of their perivitelline

SANFELICE, F. (1915). *Zbl. Bakt.* (Abt. I.) **76.** 495.
SCHEITLIN, M., SEIFERLE, E., & STUNZI, H. (1951). *Schweiz. Arch. Tierheilk.* **93.** 91.
SINIGAGLIA, G. (1913). *Pathologica.* **103.** 107.
SJOLTE, I. P. (1947). *Scand. vet. Tidsk.* **37.** 350.
STANDFUSS, R. (1908). *Arch. wiss. prakt. Tierheilk.* **34.** 109.
TIJNISSEN, G. H. B. (1952). *Tijdschr. Diergeneesk.* **77.** 507.
TREVAN, D. J. (1953). *Proc. Roy. Soc. Med.* **46.** 890.
VAN DEN AKKER, S. (1952). *Tijdschr. Diergeneesk.* **77.** 511.
VERLINDE, J. D. (1946). *Ibid.* **71.** 210.
———. (1948). *Ibid.* **73.** 922.
WATSON, E. A., & PLUMMER, P. J. G. (1942). *Amer. J. vet. Res.* **9.** 350.
WHITNEY, L. F., & WHITNEY, G. D. (1953). "The Distemper Complex." The Practical Science Publishing Co., Connecticut.
WHITTEM, J. H., & BLOOD, D. C. (1950). *Aust. vet. J.* **26.** 73.
WINQUIST, G. (1950). *Nord. vet. Med.* **2.** 367.
YOUATT, W. (1845). "The Dog." London.

spaces. On that basis they again could be divided into two groups. In the first of these the ova showed a small, semilunar perivitelline space (identical to that of Group B above), and a second group in which the ova showed a perivitelline space about double the size of that in Group B, therefore labelled as Group C (Fig. 3). While vitelline material in the ova in Groups A and B were finely granular, that of Group C showed comparatively larger vitelline globules.

Of the cleaving eggs, four were in the two-cell, one in the four-cell and one in the five-cell stage.

Two-cell stage. One of the four eggs in the two-cell stage exhibited two polar bodies (Fig. 4); the other three only contained two blastomeres. Of the two polar bodies, one appeared to be disintegrating while the second was apparently fresh and newly formed. The blastomeres were not quite equal in size and partly overlapped one another. Both were in contact with the zona pellucida. The perivitelline space contained some debris.

Four-cell ovum. In the four-cell ovum (Fig. 5) two pairs of blastomeres could be distinguished. The cells of one of the pairs were larger than those of the other, and the axes of the two pairs were perpendicular to each other. The cells were more rounded than the cells in the two-cell stage. The zona pellucida was unchanged in appearance.

Five-cell ovum. The five-cell ovum (Fig. 6) was composed of cells of different diameters. Three of them were in one plane, with one above and one below. Sperm heads and tails were embedded in the zona pellucida.

In the uterine third of the oviduct seven non-segmenting and four segmenting eggs were found. Of the non-segmenting ova three have shown perivitelline spaces similar to those in Group C, and in the remaining four eggs the vitellus was markedly shrunken and the perivitelline space was correspondingly large. The yolk globules were greatly increased in size and reduced in number. The ova were still spherical and occupied the centre space within the zona pellucida (Fig. 7).

Among the segmenting eggs recovered there was one five-, one six-, one eight-, and one ten-cell ovum. In the five-cell ovum all the five blastomeres were in one plane and were equal in size (Fig. 8). This egg was recovered with the non-segmenting one shown in Fig. 7.

Six-cell ovum. The egg in the six-cell stage was composed of six almost identical blastomeres. Five were in one plane and one was above them. There was a very minute variation in blastomere size (Fig. 9).

Eight-cell ovum. The blastomeres were of almost equal size and attached to them were two minute cells which may have been polar bodies. The blastomeres formed two planes of four cells each (Fig. 10).

Ten-cell ovum. Blastomeres of two sizes were observed but the size difference was not marked. Five of the blastomeres formed the lowest plane, four formed another one above it, and a single blastomere was on top of the latter (Fig. 11).

Eighteen undivided eggs and 21 cleaving eggs were recovered from the uterus.

While nine of the 18 undividing ova showed the shrunken vitellus and enlarged yolk globules already encountered in the oviduct, the other nine were characterised by a partly disintegrated vitelline membrane with escape of cellular material into the perivitelline space (Fig. 12).

Of the dividing eggs, two were in each of the 8-, 10-, 14- and 16-cell stages, three in the morula, and four in the blastocyst stage. Six were considered to be pathological.

The eight-cell eggs recovered from the uterus differed from those recovered from the oviduct in that they contained cells of only two sizes. These corresponded with the large and medium sized cells of the earlier ova. Small cells similar to those of the eight-cell ovum found in the oviduct were not present in this group.

Twelve-cell ovum. The blastomeres were of two sizes as in the above eight-cell ovum.

Fourteen- and 16-cell ovum. One of the 14-cell eggs contained 10 large and two small blastomeres, and two structures resembling polar bodies.

The large blastomeres were clumped together and the two small ones were situated at the periphery of the mass so formed. At the point of their attachment lay the two polar bodies similar in appearance to those illustrated in Fig. 10.

The blastomeres in the second 14-cell ovum were of equal size and no polar body was present. In the 16-cell ova the blastomeres were again similar in size and in appearance resembled the 14-cell ova.

None of the *morulae* could be photographed as such since during the examination all three of them underwent sudden structural changes—presumably due to the heat of the microscope lamp—and then presented the appearance shown in Fig. 13.

Pathological Ova

Of the six pathological ova, four were in two-cell stage and two between the 16- and 24-cell stages. In these eggs the cell outlines were clearly distinguishable and they resembled normal ova as far as shape and size are concerned. They were considered to be pathological because they appeared to be empty except for a few yolk globules (Fig. 14).

Discussion

Measurement of Sheep Ova

The diameters of the ova averaged 145μ and fell within the range of 142 to 153μ as described by Amoroso *et al.* (1942).

Zona Pellucida

Winters & Green (1935) described an alteration in the thickness of the zona pellucida in variously developed sheep ova. According to their investigation a gradual thinning takes place in the zona pellucida which corresponds to the progressive development of the ova. In the present work, however, no such alteration was found in the thickness

of the zona pellucida in ova of different developmental stages.

Corona Radiata

No corona radiata cells were attached to any of the ova recovered. This confirms the description of Assheton (1898), Allen *et al.* (1931), McKenzie & Allen (1933), Clark (1934), Green & Winters (1935) and McKenzie & Terril (1937).

Perivitelline Space

All previous authors on early sheep embryology describe a perivitelline space in the newly ovulated ovum (Assheton, 1898; Allen & McKenzie, 1931; Clark, 1934; and McKenzie & Terril, 1937) and very often a polar body (Allen & McKenzie, 1931). However, of the six eggs recovered in the ovarian third of the oviduct in my series, three had no perivitelline space.

Since Hamilton & Laing (1946) recovered ova without a perivitelline space in cattle, and regarded these as freshly ovulated eggs, it is probable therefore that the sheep ova without perivitelline space were younger eggs than any yet described.

From this series of ova it is clear that in the non-segmenting egg there is a steady increase in the size of the perivitelline space due to shrinkage of the ovum as the latter passes along the oviduct, *i.e.* as the age of the cell increases. Thus in the upper third of the oviduct the ova either did not show a perivitelline space, or at most only a comparatively narrow one. In the middle third only one ovum exhibited a perivitelline space similar to that found in the ovum from the upper third, the majority had larger spaces. Similarly most of the eggs in the uterine third showed larger perivitelline spaces than those of the middle third, whilst in the uterus the ultimate stage in the expansion of the perivitelline space is reached. This suggests a continuous shrinkage of the ova beginning almost as soon as the egg is shed.

The increase in the size of the perivitelline space is accompanied by a change in the appearance of the yolk globules which show a gradual increase in size as the ovum passes along the tube. This is accompanied by a reduction in their number, suggesting that the change may be due in part at least to coalescence.

The degenerative process terminates in the uterus in the disintegration of the ovum which is characterised by rupture of the vitelline membrane and release of the remaining globules into the perivitelline space prior to the rupture of the zona pellucida. Two ruptured zonas were recovered during this work. In one case, however, the specimen was recovered from the oviduct and it was considered that rupture in this case was due to mechanical injury.

Two-cell Stage

Clark (1934) described sheep ova in the two-cell stage and was the first to call attention to the unequal division of the cytoplasm. Hamilton & Laing (1946) recorded similar divisions in the cow's egg, and Amoroso *et al.* (1942) in that of the goat.

Trophoblastic Cells

In certain ova marked variations were noted in the size of the blastomeres (Fig. 10). Sometimes these structures were so small that they were regarded as polar bodies. Such cells were found in one eight-cell stage ovum and one 14-cell stage ovum. Similar cells were regarded by Amoroso *et al.* (1942) as polar bodies.

Some ova contained cells which were larger than those mentioned above, but were much smaller than the other blastomeres. Identical cells described by Assheton (1898) in an eight-cell ovum were regarded by him as differentiated trophoblastic cells. Clark (1934) found similar cells in a 16-cell ovum of the sheep and Heuser & Streeter (1927) in a 16-cell ovum of the pig and have regarded them as trophoblastic cells.

Correlation of Developmental Stage with the Place of Recovery

No sign of cleavage was found in the ovarian third of the oviduct. Cleaving eggs were recovered from the middle third onwards, showing an increasing number of blastomeres in proportion to the distance covered from the ovary.

Assheton (1898) and McKenzie & Terril (1937) describe the eight-cell egg as the youngest which could be recovered from the uterus. Clark (1934), however, could not find anything below the 16-cell stage.

The present series of ova show, however, that although an ovum can reach the uterus in the eight-cell stage, some reach it at a more advanced cleavage stage, *e.g.* those which were in 10- or 12-cell stages when recovered in the uterine third of the oviduct. Such variations may be due to differences in the rate at which the ovum travels along the oviduct or to variations in the rate of cleavage in the time of fertilisation.

Conclusion and Summary

1.—An account is given of 81 sheep ova recovered from the genital tract of abattoir sheep.

2.—They are grouped according to their place of recovery.

List of Figures

1.—Newly shed ovum (Group A). × 325.
2.—Ovum with moderate perivitelline space (Group B). × 325.
3.—Ovum with increased perivitelline space (Group C). × 350.
4.—Two-cell ovum. × 350.
5.—Four-cell ovum. × 350.
6.—Five-cell ovum from middle third of oviduct. × 325.
7.—Unfertilised ovum from uterine third of oviduct. × 350.
8.—Five-cell ovum from uterine third of oviduct. × 350.
9.—Six-cell ovum. × 350.
10.—Eight-cell ovum. × 350.
11.—Ten-cell ovum. × 350.
12.—Degenerated egg disintegrated vitelline membrane. × 100.
13.—Morula degenerating. × 375.
14.—" Ghost " morula.. × 350

CONTRIBUTION TO THE EARLY EMBRYOLOGY OF THE SHEEP

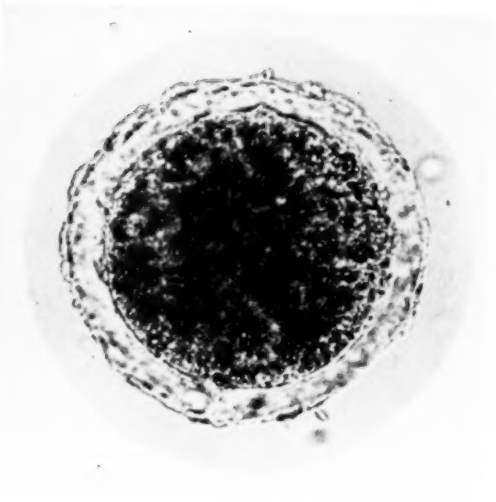

FIG. 1.

FIG. 2.

FIG. 3.

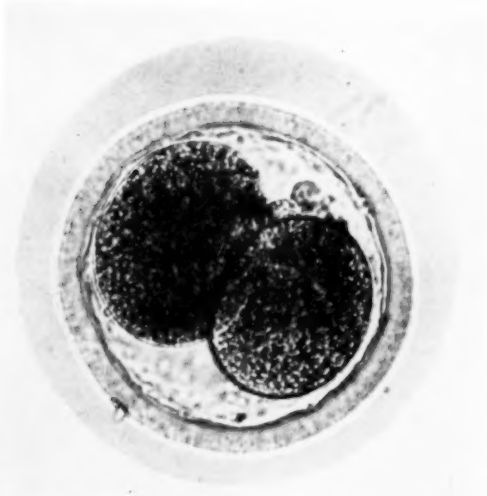

FIG. 4.

FIG. 5.

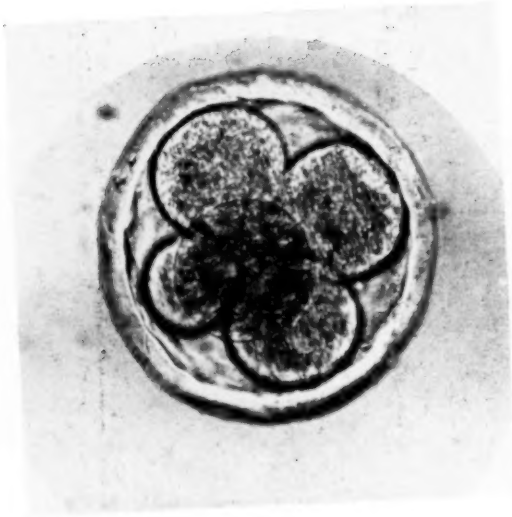

FIG. 6.

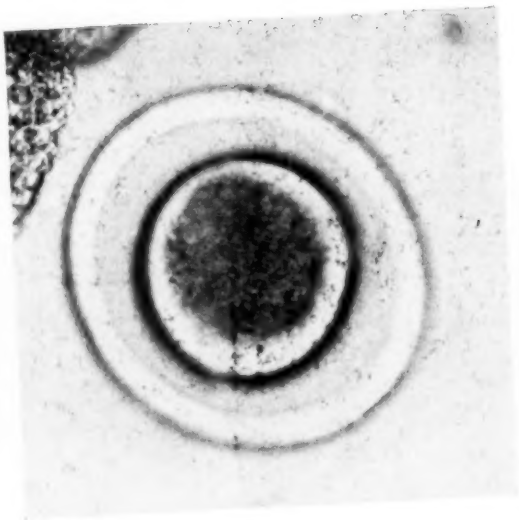

FIG. 7.

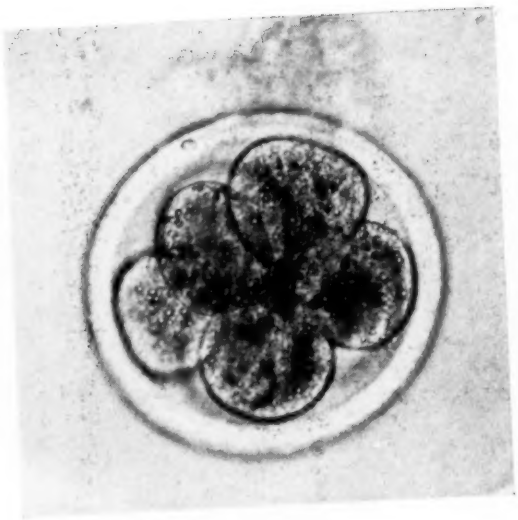

FIG. 8.

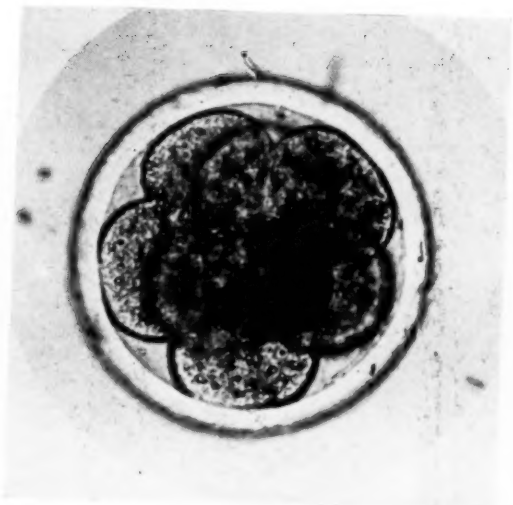

FIG. 9.

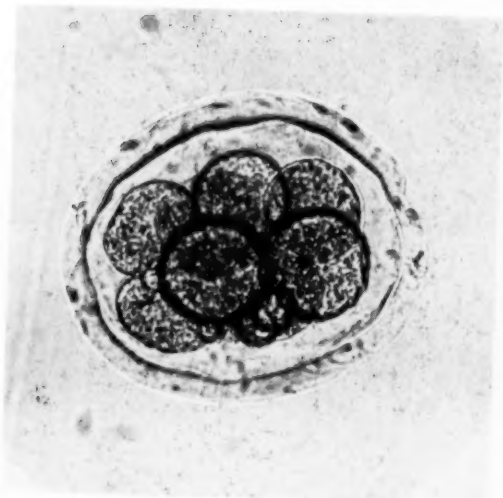

FIG. 10.

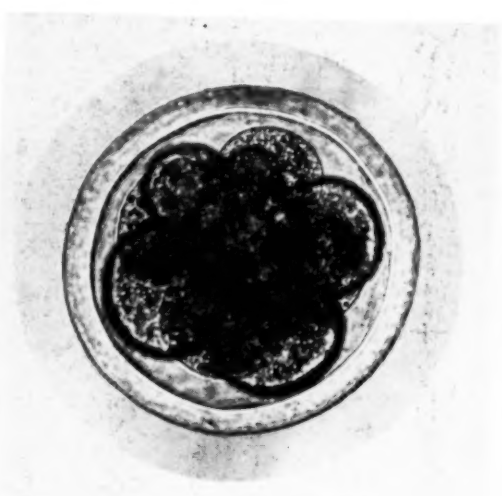

FIG. 11.

FIG. 12.

FIG. 13.

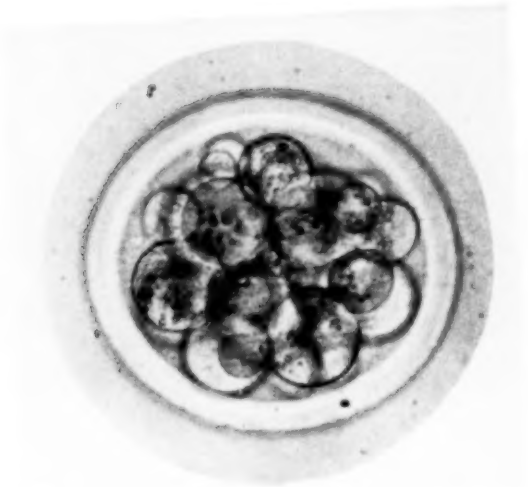

FIG. 14.

3.—Ova without perivitelline space are described and evidence presented that this appearance is characteristic of the recently ovulated egg.

4.—The perivitelline space increases in size directly as the age of the ovum.

5.—Eight- and 14-cell stages containing cells which may have been polar bodies are described.

6.—A description of some pathological ova is given.

Acknowledgment.—I have to record my indebtedness to Mr. R. N. C. Aitken, M.R.C.V.S., for his help, and to Mr. S. G. Abbott, M.B.E., M.R.C.V.S., D.V.S.M., Director of Glasgow Corporation Meat Market for facilitating the collection of all the material described in this paper.

References

ALLEN, E., & MCKENZIE, F. F. (1931). Oestrus periods, ovarian changes and tubal ova in the sheep. *Anat. Rec.* **48.** Abstract.

———, ———, KENNEDY, J. W., & BEARE, W. K. (1931). Oestrual periods, ovarian changes and tubal ova in sheep. *Ibid.* **48.** Abstract.

AMOROSO, E. C., GRIFFITH, W. F. B., & HAMILTON, W. J. (1942). The early development of the goat. *J. Anat. Lond.* **74.** 377.

ASSHETON, R. (1898). The segmentation of the ovum of the sheep, with observation on the hypothesis of a hypoblastic origin for the trophoblast. *Quart. J. micr. Sci.* **41** (new series). 205.

BAER, VON K. E. (1827). "Über Entwicklungsgeschichte der Thiere." Königsberg.

BISCHOFF, VON T. L. (1842). "Entwicklungsgeschichte der Saeugitiere und des Menschen." Leipzig.

CLARK, R. T. (1934). Studies in the physiology of reproduction in the sheep. II. The cleavage stages of the ovum. *Anat. Rec.* **60.** 135.

COLE, H. H., & MILLER, R. G. (1932). Data on reproduction in the sheep. *Ibid.* **52.** Abstract. Supplement.

GRANT, F. (1934). Studies on the physiology of reproduction in the ewe. *Trans. Roy. Soc. Ed.* **58.** 1.

GREEN, W. W., & WINTERS, L. M. (1935). Studies on physiology of reproduction in the sheep. III. The time of ovulation and the rate of sperm travel. *Anat. Rec.* **61.** 457

HAMILTON, W. J., & LAING, J. A. (1946). Development of the egg of the sow up to the stage of blastocyst formation. *J. Anat. Lond.* **80.** 194.

HAUSMANN, U. F. (1840). "Ueber Zeugung und Entstehung des wahren weiblichen Eies bei den Säugethieren und dem Menschen." Hannover.

HEUSER, C. H., & STREETER, G. H. (1927). Early stages in the development of pig embryos, from the period of initial cleavage to the time of appearance of limb-buds. Contrib. to *Embryol. Carneg. Instn.* **20.** 1.

KELLEY, R. B. (1937). "Studies in fertility of sheep." Commonwealth of Australia Counc. for Sci. & Indust. Res. Bull. No. 112.

MCKENZIE, F. F., & ALLEN, E. (1933). "Ovulation and tubal ova in the ewe." *Miss. Agr. Exp. Sta. (Res.) Bull.* 328.

——— & TERRIL, C. E. (1935). The ovulation in the ewe. *Anat. Rec.* **61.** 58. (Supplement.)

——— & ———. (1937). "Oestrus, ovulation and related phenomena in the ewe." Miss. Agr. Exp. Sta. Res. Bull. No. 264.

CLINICAL COMMUNICATION

The Prevention and Treatment of Sepsis in the Bovine Uterus with Bis-parachlorphenyldiguanidohexane

J. McWILLIAM
Nantwich

THE effective control of uterine infection in the bovine is one of the major problems in general veterinary practice. This clinical paper describes an assessment under field conditions of a new synthetic antibacterial compound 1:6-di-4′-chlorophenyldiguanidohexane (Compound 10,040).

Preliminary information on Compound No. 10,040 has been published elsewhere. Laboratory tests showed that even in the presence of blood, *Staph. aureus, Bact. coli, Ps. pyocyanea, Strep. pyogenes* and *Strep. faecalis* are rapidly killed by 1:1,000. Growth of these organisms is prevented by 1:10,000 to 1:800,000 of the drug and toxicity is very low indeed. Preliminary trials indicated a high degree of activity in the uterus and various formulations involving the use of almost insoluble salts to give prolonged action were tested. A formulation containing the Compound in an effervescent base permitting its use as a pessary or as a suspension for use with a catheter was finally adopted.

Over the last two years it has been used in a variety of uterine conditions. The following case histories describe the clinical results achieved in various pathological states of the bovine uterus.

4.—"Hibitane*" in Caesarean Section in the Bovine

Subject

Shorthorn cow. Third calf.

History

The cow had been uneasy for 24 hours and examination showed a partly dilated os (two fingers). At examination 24 hours later no change was observed except that the membranes had ruptured. On the third day a stinking discharge was present and the os dilated three fingers in breadth.

Treatment

Caesarian section by the normal left flank procedure was undertaken. The uterine wall was thickened and a markedly distended calf removed with stinking foetal membranes and exudate, some of which escaped on to the abdominal wall and a little into the abdominal cavity. The uterus was extremely difficult to suture owing to the friable nature of its wall, leading to the tearing out of

* "Hibitane"—Registered trade mark of Imperial Chemical (Pharmaceuticals), Limited.

1.—The Prevention of Metritis where the Placenta has been Retained. The Series in Table I is Typical of Over 100 Cases Similarly Treated

TABLE I

Subject	History	Initial treatment	Subsequent treatment	Subsequent history and general remarks
(i) Friesian cow, aged five years.	21.5.52. Aborted six months foetus. Membranes typical of brucellosis. No marked discharge.	22.5.52. Two pessaries inserted deep into uterus and 25 mg. Stilboestrol injected intramuscularly.	25.5.52. Membranes removed manually. Cow feeding normally. No "smell" or marked discharge. 2.6.52. Two more pessaries inserted. Uterus involuted. No discharge.	No "whites" developed. Cow conceived at first service.
(ii) Friesian cow, aged five years (same farm as above).	28.5.52. Aborted seven months foetus. Membranes typical of brucellosis. Fair discharge. No fetid smell.	30.5.52. Two pessaries inserted and 25 mg. Stilboestrol injected intramuscularly.	2.6.52. Membranes naturally voided. Examination showed little fluid in uterus and there was no putrefactive odour. Two pessaries inserted.	Owner reported no "whites" developed and cow conceived at first service.
(iii) Ayrshire cow, aged eight years.	8.7.53. Normal parturition.	10.7.53. Placenta firmly attached with some uterine fluid and slight smell. No sign of acute metritis. Two pessaries inserted and 25 mg. Stilboestrol injected intramuscularly.	13.7.53. Placenta easily removed. Fair amount of uterine exudate but no offensive smell. Uterine wall flexible and not inflamed. Temperature normal. Two pessaries inserted.	Uneventful recovery with no subsequent "whites."
(iv) Ayrshire cow, aged six years.	8.9.53. Calved at full term after fairly prolonged labour.	11.9.53. Temperature normal, placenta firmly attached. Some little fluid present in uterus, little smell. Two pessaries inserted and 30 mg. Stilboestrol injected intramuscularly.	14.9.53. Temperature 102° F., placenta still attached. Rather more fluid in uterus, little smell. Two pessaries inserted. 17.9.53. Temperature 102° F. Placenta had come away. Fair amount of fluid syphoned from uterus with little smell. Two pessaries inserted through os.	Uninterrupted recovery with no endometritis.
(v) Friesian cow, aged six years.	8.11.53. Calved full-term twins. Part only of placenta voided.	10.11.53. Normal temperature. No fetid smell. Two pessaries inserted and 30 mg. Stilboestrol injected intramuscularly.	12.11.53. Removal of placenta not attempted. Some fluid present but no smell. Two pessaries inserted. 14.11.53. Placenta lying free in uterus and was removed. Some fluid with a slight smell syphoned off. Two pessaries inserted.	Ten days later owner reported appearance of slight white discharge. This ceased in a few days.
(vi) Guernsey cow, aged eight years.	11.11.53. Calved full time but the calf was still-born.	14.11.53. No uterine smell. Two pessaries inserted and 30 mg. Stilboestrol injected intramuscularly.	17.11.53. Placenta easily removed. Some fluid in uterine but no offensive odour. Fluid syphoned off. Two pessaries inserted.	Owner reported an uninterrupted recovery with no subsequent "whites."

stitches, etc. Four pessaries were placed in the uterus before completion of suturing, and the uterine cavity and wall were swabbed as much as possible. Two m.u. penicillin were applied to the abdominal cavity. Compound 10,040 powder was dusted over the abdominal wall. Suturing was in three layers—peritoneum and deeper muscles, subcutaneous tissue and upper muscle layer, and skin. The compound was dusted on each layer.

Sequel

The cow was fairly ill for eight days, during which time a course of penicillin injections was given. The abdominal wall wound healed by first intention, except for a small area at the lower part where a suture was removed and some purulent fluid drained. Uterine discharges ceased in seven days when rectal examination showed the uterus to be fairly well involuted. After 10 days the cow was progressing satisfactorily. A certain amount of "whites" developed. No attempt was made to milk the cow, which was sold fat about four months later.

Subsequently a series of five caesarian sections was performed on cows, comparing the local application of Compound 10,040 and penicillin. These were carried out for varying reasons—foetal monster, over-large calf (twice), non-dilatation of os (twice). Operative technique was as in the above case. In each case membranes were easily removed with the foetus. In three cases the compound was inserted into the uterus and dusted on the flank wound. In these cases the flank wall healed by first insertion. The uterus involuted normally with no more than the normal post-parturient discharges. In two cases 300,000 units of penicillin were inserted into the

2.—The Treatment of Acute Metritis as a Complication of Retained Placenta. The Examples in Table II are taken from 50 Similar Cases Treated with Compound 10,040

TABLE II

Subject	History	Initial treatment	Subsequent treatment	Subsequent history and general remarks
(i) Ayrshire cow, aged six years.	7.7.53. Calved full time.	11.7.53. Temperature 103° F. Slightly off food. Placenta fairly firmly attached. No attempt at removal. Good deal of offensive uterine fluid present. Two pessaries inserted and 20 mg. Stilboestrol intramuscularly.	13.7.53. Temperature 103° F. Feeding fairly well. Placenta easily removed. Fluid syphoned from uterus, no more offensive than previously. Two pessaries inserted. 16.7.53. Temperature 102° F. Appetite good. No uterine discharge or offensive smell present.	Owner reported normal recovery with no subsequent " whites."
(ii) Shorthorn cow, aged seven years.	17.7.53. Twin calves at full term.	20.7.53. Temperature 105° F. Off food. Half of placenta easily removed. A good deal of offensive fluid was present in the uterus. Three pessaries inserted. 30 mg. of Stilboestrol and 2 m.u. penicillin injected intramuscularly.	22.7.53. Temperature 103° F. Eating fairly well. Uterine fluid less offensive. Three pessaries inserted, 2 m.u. penicillin injected intramuscularly. 24.7.53. Temperature 103° F. Eating fairly well. Remainder of placenta removed, and a good deal of exudate syphoned off. Two pessaries inserted.	Owner reported uneventful recovery with discharge ceasing after 48 hours. A week later " whites " developed which cleared up after a few days.
(iii) Ayrshire cow, aged five years.	17.11.53. Owner believed cow to have " cleansed."	22.11.53. Cow off food. Straining. Temperature 104° F. Os uteri almost completely closed. Stinking exudate. Three pessaries inserted together with intramuscular injection of 30 mg. Stilboestrol and 2 m.u. penicillin.	24.11.53. Temperature 103° F. Cow feeding. Copious uterine discharge. Two pessaries inserted. 26.11.53. Temperature 102° F. Cow feeding. Little uterine discharge with no offensive smell. Two pessaries inserted.	Uneventful recovery two weeks later cow treated for a mild endometritis.
(iv) Friesian cow, aged four years.	18.10.53. Calved full term.	21.10.53. Cow off food, temperature 103·6° F. Placenta firmly attached with rather offensive odour. Two pessaries inserted but no other treatment.	23.10.53. Two pessaries, left with owner, inserted. 25.10.53. Cow feeding and temperature 102·2° F. Placenta easily removed. A good deal of fluid present in the uterus with only slight odour. Two pessaries inserted after syphoning off exudate.	Uneventful recovery with no endometritis.
(v) Shorthorn cow, aged six years.	12.12.53. Normal calving.	16.12.53. Cow off food with temperature of 104° F. Marked metritis with stinking fluid in uterus. Three pessaries inserted and 30 mg. Stilboestrol intramuscularly.	19.12.53. Placenta easily removed. Exudate much less offensive and syphoned off. Two pessaries inserted.	Owner reported all smell disappeared after two days, and discharge ceased three days later.

uterus, and the flank wound was dressed with penicillin in oil. In these two cases the bottom of the wounds healed after some abscess formation, while uterine discharges continued for some time.

5.—Treatment of Vaginal Laceration Occurring at Parturition

Subject

Friesian two-and-a-half-year-old heifer.

History

Following a difficult parturition due to big calf, three " tears " were found in the vaginal wall with a good deal of bruising. The placenta followed immediately after the calf.

Treatment

17.6.53.—Two pessaries were placed in the uterus and the owner was given pessaries to insert into the vagina daily. 20.6.53.—A certain amount of swelling of the vulva was seen. The owner reported this to have decreased markedly from that present the day after parturition. There was little discharge in the vagina and no necrosis of the vaginal wall. The wounds were granulating. 23.6.53.—The wounds had practically healed with no necrosis.

The above was typical of 12 recorded cases.

Discussion

The assessment of the effectiveness of any agent used in the prevention and control of sepsis is always difficult. The possible errors are always increased under conditions, such as general practice, where fully controlled trials are not possible. However, by accurately recording a large number of field cases a satisfactorily clinical evaluation can be attained.

3.—The Treatment of Endometritis. The Response to Treatment of the Eight Animals is Shown in Table III. These Records are taken from 200 in the Same Series

TABLE III

Subject and history	Initial treatment	Subsequent treatment	Subsequent history and general remarks
(i) Two Ayrshire cows and one heifer. All had been calved three to five weeks and showed mild endometritis with a purulent discharge. The uterus was slightly thickened but contained no great amount of fluid.	5.12.53. One pessary suspended in 8 oz. of water injected by catheter per os uteri, in each case.	6/7.12.53. Owner reported good deal of discharge. None seen later. 14.12.53. Rectal examination showed uterus almost normal. No exudate in vagina.	Owner reported conception at first service in two cases and at second service in one case.
(ii) Three Friesian cows, calved between three and four weeks. The placentae had been retained for two and four days respectively in two animals. The uteri were slightly thickened and a fair amount of purulent discharge was present.	4.11.53. One pessary in 8 oz. of water injected by catheter in each case.	13.3.53. Two showed clear uterine discharges and the third a few small clots in clear mucous exudate. This cow was re-treated.	No discharge seen after a further two days.
(iii) Two Ayrshire cows. Both had been calved about four weeks and had retained the placenta for four and six days respectively. There was a marked purulent discharge and the uterine horns were distended with pus.	4.11.53. One pessary in 8 oz. of water injected into each uterus.	11.11.53. Marked discharge shown a few days previously but little decrease in uterine contents observed on examination. A further pessary suspension injected. The treatment was repeated weekly on four occasions, Stilboestrol also being given intramuscularly. The exudate decreased steadily in quantity.	12.12.53. Uteri slightly thickened. No purulent discharge.

(*a*) Prevention of Metritis in Retained Placenta

Over 100 cases were recorded.

In only three cases did a metritis develop and that to a mild degree. In each of the three a slightly fetid odour was present when treatment began. One treatment with Compound 10,040 proved to be effective in preventing metritis developing for at least two to three days, and in practice, treatment every third day proved successful. In the majority of cases no subsequent endometritis developed. The drug proved in our hands to be much more effective, and much longer lasting, than either penicillin or the sulphonamides in the above cases.

(*b*) Treatment of Metritis

Over 500 cases were recorded.

Every animal treated recovered. In the majority of cases two treatments were sufficient to clear up the condition. Again Compound 10,040 proved to be more effective than penicillin or the sulphonamides.

(*c*) Treatment of Endometritis

Over 200 cases were recorded.

In mild cases one uterine douche was sufficient to clear up the condition. More severe cases required two and even three douches at weekly intervals. In pyometra, as could be expected, little response was evoked until evacuation of most of the pus had been effected by stilboestrol.

Varying strengths of solution were used from approximately one-quarter pessary suspended in 8 oz. water to one pessary in the same amount—this latter strength seemed to be more effective. A little straining was noted in a few cases following douching.

Results were much more satisfactory than those previously obtained with iodine solution.

(*d*) Treatment of Vaginal Laceration

Twelve cases were recorded.

Treatment was most successful in the prevention of vaginal wall necrosis. Healing was much more rapid than with older methods of vaginal douches.

Summary

The use of a new compound 1:6-di-4′-chlorophenyldiguanidohexane in preventing and treating uterine sepsis in over 360 bovines is described. The results indicate an improved control of infection over that obtained with penicillin and sulphonamides, and irrigation with iodine.

Acknowledgments.—The author wishes to record his thanks to Mavis J. Francis, M.SC., M.R.C.V.S., and T. W. Groves, B.SC., M.R.C.V.S. (Wilmslow) for advice and assistance, and also to Imperial Chemical (Pharmaceuticals) Ltd. for supplies of " Hibitane " formulations.

REFERENCE

DAVIES, G. E., FRANCIS, J., MARTIN, A. R., ROSE, F. L., & SWAIN, G. (1954). 1 : 6-di-4¹-chlorophenyldiguanidohexane (" Hibitane "). Laboratory Investigation of a New Antibacterial Agent of High Potency. *Brit. J. Pharmacol.* **9**. 192.

News and Comment

OFFICIAL OPENING OF THE ROYAL VETERINARY COLLEGE AND HOSPITAL

The official opening of the 163rd Session was held at the Royal Veterinary College on Monday, October 4th, 1954, when the prizes for the previous session were presented by Sir Cecil P. G. Wakeley, BART., K.B.E., C.B., D.SC., F.R.C.S., Immediate Past President of the Royal College of Surgeons (England), in the presence of a distinguished gathering which included representatives of the Governing Body and of the University of London.

Lieut.-Col. Sir Merrik Burrell, BART. (Senior Vice-President of the College) presided, supported by the Acting Principal, Professor J. McCunn. With them on the platform were Dr. W. R. Wooldridge, President of the Royal College of Veterinary Surgeons, and Professor G. H. Wooldridge, Past President of the Royal College. Several Officers of the University of London were present, including Mr. J. R. Stewart (Clerk of the Court), Mr. J. Hood Phillips (Secretary of the Senate), Mr. J. D. Angus (Deputy Clerk of the Court), Mr. A. Tattersall (Deputy Academic Registrar). Other Officers of the Royal College who attended were Mr. G. N. Gould (Treasurer), Mr. W. G. R. Oates (Secretary and Registrar), Mr. C. W. Francis and Mr. H. M. Anderson.

List of Prizewinners and Awards

FINAL YEAR

Nestel, Heather M., Coleman, Silver; R.A.S.E., Bronze; Clinical, Bronze. Nestel, B. L., R.A.S.E., Silver; Cecil Aldin, First; Centenary, Silver; Medicine, Silver. Holmes, R. G., Cecil Aldin, Second; Surgery, Silver. Olorenshaw, P. H., Clinical, Silver; Bywater, J. E. C., Coleman, Bronze. Benbow, G. M., Medicine, Bronze; Surgery, Bronze.

FOURTH YEAR

Derbyshire, J. B., Tompson, First; Evans Final Year Studentship; Clinical, Silver; Pathology, Bronze. Down, B. C., Centenary, Silver; Clinical, Silver. Taylor, E. L. G., Tompson, Second. Bedson, I. F., Pathology, Silver. Gray, J., B.O.C.M., Animal Husbandry Prize.

THIRD YEAR

Prasad, A. K., Centenary, Silver. Oakley, G. A., Clinical, Silver. Oke, M. A., Clinical, Silver. Hollands, J. G., Hygiene, Silver; Pharmacology, Bronze. Gill, E. A., Pharmacology, Silver; Hygiene, Bronze.

FIRST YEAR

Creek, M. J., Centenary, Silver; Chemistry, Silver; Physics, Bronze. Seed, J. H., Physics, Silver. Wade, D. W. H., Biology, Silver. Hitchcock, P. L., Chemistry, Bronze. Prosser, G. G., Biology, Bronze.

SWINE FEVER—REGISTERED VACCINATED HERDS SCHEME

It has come to the notice of the Association that veterinary surgeons may not always have realised that under the Swine Fever—Registered Vaccinated Herds Scheme, the vaccine used must be crystal violet vaccine issued from Weybridge. We understand from the Ministry of Agriculture that there have been instances where other vaccines have been used. Veterinary surgeons are reminded of their obligation to use Weybridge vaccine and that the Ministry must take a serious view of any failure to observe this requirement.

ACCESSIONS TO THE R.C.V.S. LIBRARY

The following books have been received in the library during the last week.

DYKSTRA, R. R. Veterinary medicine in Kansas. Manhattan, Kansas State College, 1952.

ENGELBRETH-HOLM, J. Spontaneous and experimental leukaemia in animals, Edinburgh, Oliver & Boyd, 1942.

HAMMOND, J. *Editor.* Progress in the physiology of farm animals, three vols. London, Butterworth, 1954. So far only Vol. I has been published.

KOCH, W. *and* DEIMEL, B. Uber Krankheiten der Menschaffen. Jena, Gustav Fischer, 1952.

GUTHRIE, D. A history of medicine, London, Nelson, 1945.

MARKOWITZ, J. Experimental surgery, including surgical physiology. *Third edition.* Baltimore, Williams, Wilkins Co., 1954.

SANDOZ LTD. Sandoz atlas of haematology. Basle, Sandoz Ltd., 1952.

STURKIE, P. D. Avian physiology, Ithaca, Comstock publishing associates, 1954.

WHITNEY, L. F. The complete book of cat care. London, Gollancz, 1954.

WOOLDRIDGE, W. R. Farm animals in health and disease. London, Crosby Lockwood, 1954.

The Librarian would be pleased to hear from anyone who has a copy of Sir Frederick Smith's Early History of Veterinary Literature *Volume I*, which they would be willing to part with, and also the R.C.V.S. Annual Reports for 1911/12 and 1920/21 or the Registers in which they were printed, 1913 and 1922 respectively.

THE ROYAL COLLEGE OF VETERINARY SURGEONS

On Friday, October 8th, 1954, the President of the Royal College of Veterinary Surgeons, supported by the Officers and members in the field of veterinary education, gave a small sherry party at the Royal College. Amongst those who accepted invitations were certain Vice-Chancellors of Universities having Veterinary Schools, the Chairman of the University Grants Committee, and the Clerk to the Privy Council.

BRISTOL UNIVERSITY VETERINARY SCHOOL

Veterinary history was added to at Bristol on Monday, October 11th, 1954, when in a special Degree Congregation the Vice-Chancellor, Sir Phillip Morris, C.B.E., before a distinguished gathering admitted the first veterinary students of the University to the Degree of Bachelor of Veterinary Science. There were 19 graduates, three with Second-Class Honours.

Immediately following the Congregation, the new graduates were admitted as members of the Royal College of Veterinary Surgeons by the President, Dr. W. R. Wooldridge.

In celebration of this historic occasion a luncheon was given by the Vice-Chancellor for representatives of the University and the Royal College.

A fuller report will appear in our next issue.

COMING EVENTS

Oct. 21st.—Ordinary General Meeting of the South-Eastern Division, B.V.A., at the Royal Star Hotel, Maidstone, 2.30 p.m.

Oct. 22nd.—Annual Dinner Dance of the South Wales Division, B.V.A., at the Seabank Hotel, Porthcawl, 7.30 p.m.

Oct. 22nd.—Meeting of the South-East Midlands Division, B.V.A., at Franklin's Garden Hotel, Northampton, 7 p.m.

Oct. 28th.—General Meeting of the Herts and Beds Division, B.V.A., at 70, Holywell Hill, St. Albans, 7.30 p.m.

Oct. 28th.—General Meeting of the Society of Practising Veterinary Surgeons, B.V.A., at the Grand Hotel, Peterborough, 2.30 p.m.

Oct. 29th.—Informal Dinner for the Lancashire Division, B.V.A., at the Blue Cap Restaurant, Sandiway, Cheshire, 7.15 p.m.

Oct. 29th.—Meeting of the Yorkshire Division, B.V.A., in the Hotel Metropole, King Street, Leeds, 1, 2.30 p.m.

Nov. 3rd.—General Meeting of the Scottish Metropolitan Division, British Veterinary Association at the Royal (Dick) School of Veterinary Studies, 2.30 p.m.

Nov. 16th & 17th.—B.V.A. Conference on the Feeding of Farm Livestock for Health and Production in the South Hall, Victoria Halls, Bloomsbury Square, London, W.C.1. Admission by ticket only. Please apply not later than November 8th, on form published with issue of October 9th.

Dec. 3rd.—Annual Dinner and Dance of the North Wales Division, B.V.A., in the Imperial Hotel, Llandudno.

NOVEMBER MEETINGS OF COMMITTEES AND COUNCIL OF THE B.V.A.

AT NO. 7, MANSFIELD STREET, W.1.

Wednesday, November 3rd.—

11 a.m. Veterinary State Medicine Committee.
2.30 p.m. Parliamentary & Public Relations Committee.
4 p.m. Home Appointments Committee.

Thursday, November 4th.—

10.30 a.m. Organising Committee.
12 noon Finance Sub-committee.
2 p.m. General Purposes & Finance Committee.

AT CONNAUGHT ROOMS, GREAT QUEEN STREET, W.C.1.

Friday, November 5th.—

10.30 a.m. Council Meeting.

PERSONAL

Births

BLYTH.—On October 6th, 1954, at W.J.C. Hospital, Braintree, to Elizabeth (*née* Aitken), M.R.C.V.S., and Anthony Blyth, M.R.C.V.S., a daughter—Sarah.

LEMON.—On October 4th, 1954, at The Borough General Hospital, Ipswich, to Vivien (*née* Kipling), wife of D. A. Lemon, M.R.C.V.S., a daughter.

LEWIS.—On September 30th, 1954, at 24, Vincent Avenue, Stratford-on-Avon, to Judy and Michael Lewis, M.R.C.V.S., a brother for Kevin, Sue and Jenny.

JOHN GIBSON, M.R.C.V.S.

Mr. W. Thomson writes:—The death of John Gibson, M.R.C.V.S., of Dundee, leaves still another gap in the ranks of those practitioners who have seen practice change so markedly in the last half century. Always active, he had been in practice in Dundee for the past 40 years, and was held in high esteem by both colleagues and friends. One could have nothing but admiration for his honesty of purpose, conscientiousness and devotion to duty. When I was a student with him, and later when I became his partner, his patience, quick wit and ever-helping hand made light of the day's work.

He was for many years a member of the Scottish Metropolitan Division; he attended its meetings whenever possible and took a keen interest in the proceedings. He was a member of Dundee Canine Club, and Lochee & District Canine Club, serving them officially as well as showing his own dogs. Outside of the profession his interests lay in the theatre and in Rotary, and he was a Past President of the Dundee Rotary Club.

We have lost a colleague who gave his best to the profession. To Mrs. Gibson and their son we extend our deepest sympathy.

ADDRESSES OF DISEASE-INFECTED PREMISES

This list given below indicates, first, the county in which are situated the premises on which disease has been confirmed, followed by the postal address and date of outbreak.

Anthrax :

Angus. Nether Careston, Careston, Brechin (Oct. 5).

Cumberland. Balladoyle Farm, Silloth, Carlisle (Oct. 5); Beech House, Cumwhinton, Carlisle (Oct. 6).

Yorks. Throstle Nest Farm, Aysgarth, Leyburn (Oct. 6).

Fowl Pest:

Hants. Old Manor Farm, Blackwater (Oct. 5).

Northants. Bank Yard, North Street, 78, North Street; and 76, North Street, Crowland, Peterborough (Oct. 6).

Sussex. Winteregg Farm, Eastergate Lane, Walberton, Arundel (Oct. 4).

Swine Fever:

Ches. Valley Farm, Goostrey, Crewe; Higher Farm, Threaphurst Lane, Hazel Grove, Stockport (Oct. 6).

Essex. Bower Farm, Bower Farm Road, Havering, Romford (Oct. 4); Gladwyn's Farm, Sheering, Harlow (Oct. 8).

Glamorgan. Piggeries at Pengram Allotments, Cardiff; Fairview Holding, New Cardiff Road, Cwmbach, Aberdare (Oct. 4); Pengram Allotments, Cardiff (Oct. 8).

Glouc.s. Hengaston Farm, Cam, Dursley (Oct. 4).

Herts. Hope Cottage, Flamstead End, Cheshunt, Waltham Cross (Oct. 4).

Hunts. Shooters Hollow, Buckden (Oct. 4).

Kent. Barnet Villa, The Drift, Croydon Road, Keston, Bromley (Oct. 4); Chalk Hole, Canterbury Lane, Upchurch, Sittingbourne (Oct. 5); Upper Cobham Farm, Lenham, Maidstone (Oct. 7).

Lancs. Bugle Hall Farm, Hindley Road, West Houghton, Bolton; Hilltop Farm, Southport Road, Lydiate, Maghull, Liverpool (Oct. 4); Elm Farm, High Street, Newton-le-Willows (Oct. 5); Park Lane Farms, Pendlebury, Swinton (Oct. 7).

Lincs. Laburnum Cottage, Wilsford, Grantham; Stone House Farm, Burton Road, Lincoln (Oct. 4).

London. Courtland Nurseries, Courtlands Avenue, Lee, S.E.12 (Oct. 8).

Salop. South Enden Farm, Chetton, Bridgnorth (Oct. 8).

Somerset. Spring Farm, East Dundry (Oct. 5).

Suffolk. "Sunset," Alpheton, Sudbury (Oct. 7).

Worcs. "Cader-Idris," Ullenhall Lane, Beoley, Redditch (Oct. 4); Ambrose Farm, Lower Broadheath, Worcester (Oct. 8).

Yorks. Bogg Hall Farm, Coneysthorpe (Oct. 4); Colt Green Farm, Wortley, Sheffield (Oct. 9).

CORRESPONDENCE

The views expressed in letters addressed to the Editor represent the personal opinions of the writer only and their publication does not imply endorsement by the B.V.A.

A LEGITIMATE GRIEVANCE

Sir,—May I add my support to many of Mr. Humphrey's remarks in this week's (September 18th) issue of THE RECORD? I also am employed in a clinic in the Brighton area and it seems to me that reorganisation and redistribution of clinics would benefit, not only the profession, but also our patients and their owners as well.

In this area at least four societies provide clinic services of one kind or another—it is obvious that money, at present spent on four different administrations, could, if pooled, provide a wonderful hospital service 24 hours a day, both for poor people and for the use of local veterinary surgeons. The facilities of this hospital are already used, with goodwill on all sides, by several local veterinary surgeons, and I am sure there would be an even greater demand if it was properly organised on a paying and economic basis.

A free service for poor persons is definitely necessary—I disagree with those who say there are no poor people nowadays. There are many—particularly old age pensioners and others—who may be able to afford occasional treatment for their animals but not the expense of an operation or a long illness. I know that many of my colleagues often help such people, but often they are too proud to accept charity of this kind.

There are, however, as we all know, a great many people using clinics who do not qualify. Some of these are the chronic dissatisfied grumblers who probably owe bills to every veterinary surgeon in town. Some, however, do genuinely feel that it seems hard luck that because they are honest enough to offer payment for their animals they are denied facilities which they may not be able to get elsewhere. In the many "brushes" which I have with would-be clients I find these latter the most difficult to answer.

On the question of almoners, a good deal could be said. It seems to me that unless almoners are trained and employed by some body other than the society running the clinic, efficient almoning is most unlikely—dissatisfied people who have been turned away are all too prone to stirring up trouble in headquarters!

Yours faithfully,

ARTHUR WELCH.

4, Robertson Road, Brighton, 5, Sussex.

September 19th, 1954.

GAMMA GLOBULIN PROPHYLAXIS OF CANINE VIRUS DISTEMPER

Sir,—With reference to the above article which appeared in your issue of September 18th, 1954, I beg the courtesy of your columns to ask for further details of the experimental work reported by Messrs. Larin and Hodgman. Three significant aspects of the work would appear to require further elucidation.

Firstly, there is no indication of the potency or dosage of the gamma globulin employed, nor are details given of the methods used in obtaining the immune serum from which the material was prepared. It is not stated whether the preparation was homologous or heterologous or whether any control

dogs were injected with " normal " gamma globulin and virus. Secondly, no indication is given of the infective titre or of the strain or group (Larin & Hodgman, 1954) of the distemper virus used. Also, the number of animals given virus alone is not stated. Thirdly — and particularly important—it is not shown whether, in the " well-controlled " experiment involving 400 dogs, the subjects were challenged with virulent distemper virus. If so, what strain and what dosage and at what interval after immunisation the challenging dose was administered ? It would seem to be necessary to be in possession of the foregoing data before a critical appraisal of the value of this method can be reached.

Exception might be taken to the statement that gamma globulin can be made bivalent or even multivalent. It is obvious that the preparation is no more bivalent or multivalent than is the antiserum or are the antisera from which it is obtained. This being so it is difficult to see in what fundamental respect the suggested method differs from the " serum-virus " method of Laidlaw and Dunkin (1931). Concentrated gamma globulin preparations undoubtedly offer many advantages over the use of antiserum itself, and these have been summarised by the authors. Although Larin and Hodgman have made no comparison of the two methods, manifestly such comparisons will be drawn. Anyone wishing to do so would be well advised to refer to the stringent criteria adopted by Laidlaw and Dunkin in assessing the value of their product.

Yours faithfully,

J. M. MACKAY,

Department of Veterinary Pathology,
University of Glasgow Veterinary School,
85, Buccleuch Street, Glasgow, C.3.

September 20th, 1954.

References

LAIDLAW, P. P., & DUNKIN, C. W. (1931). *J. comp. Path. & Therap.* **44.** 1.

LARIN, N. M., & Hodgman, S. F. J. (1954). *Vet. Rec.* **66.** 339.

CORTICAL ACTIVITY IN ELECTROCUTED DOGS

Sir,—I was greatly interested in Dr. Roberts's excellent paper on Cortical Activity in Electrocuted Dogs in THE VETERINARY RECORD of September 25th, 1954. His ingenious methods will be appreciated by all electro-physiologists who have worked in this difficult field, and his results have been of such general interest as to make headline news in the daily papers.

Nevertheless, I am disturbed by some aspects of this work. Study of the references given by Dr. Roberts shows that his work confirmed conclusions reached by other physiologists in 1934 and 1938. These earlier papers were brought to the notice of the B.V.A. more than 18 months ago, and science usually progresses by assuming the results of reputable research workers to be correct. Dr. Roberts's work involved experiments which caused pain to a number of dogs, and which were not pleasant for the experimenter.

Would it not have been better, 18 months ago, if the B.V.A. had accepted the results of previous workers in this field, and devoted its resources to the development of a more humane electrocution cabinet? Animal Welfare societies are not likely to be grateful when apparatus is condemned and no substitute is available; neither is the reputation of the B.V.A. increased, in the eyes of scientists or anti-vivisectionists, by its sponsorship of redundant investigations.

Yours faithfully,

Fairmead, PHYLLIS G. CROFT,
High Beech, Loughton, Essex.

September 26th, 1954.

EDITORIAL NOTE

Dr. Croft implies that the conclusions which Dr. Roberts has drawn about the state of unconsciousness of electrocuted dogs had already been reached by Simpson & Darbyshire in 1934, and by Sugar & Gerard in 1938, and their information was available to the B.V.A. 18 months ago. This is not the case. These earlier papers are concerned only with the effects of circulatory arrest in anaesthetised animals and the findings reported in them acquired relevance to the present investigation only after it had been shown that the shock did not itself produce any change in the electrical pattern of cortical activity. Until this had been demonstrated it was still possible to hold, as Hume suggested in 1950, that electrocution might produce unconsciousness rapidly by inducing " pain shock " and that, if the onset of unconsciousness was sufficiently rapid, the method could be regarded as humane.

It is for this reason that the B.V.A.'s support for the electrocution procedure was not withdrawn until some definite information became available in the form of Dr. Roberts's results.

A great deal of thought and experimentation has gone into the problem of devising alternative procedure and a revised recommendation will shortly be issued.

Reference

HUME, C. W. (1950). *Vet. Rec.* **62.** 811. "Electrocution of Unwanted Dogs and Cats."

OUTBREAKS OF SWINE FEVER ON THE EARL OF SHREWSBURY'S ESTATE

Sir,—With regard to the Statement issued by the Ministry of Agriculture and Fisheries and printed under the above heading in your issue of September 25th, 1954, may I make it quite clear to the members of the veterinary profession that the vaccine of " Foreign Origin " referred to in this statement was not the " Behring " Crystal Violet Swine Fever Vaccine which is distributed in this country by International Serum Co. Ltd., a fact which has been confirmed in writing by the veterinary surgeon who attended the respective herd.

Yours faithfully,

H. ENOCH,
pp. International Serum Co. Ltd.